Volume 12 Number 6 **12 July 2025**

The Taxonomic Report

OF THE INTERNATIONAL LEPIDOPTERA SURVEY

ISSN 2643-4776 (print) / ISSN 2643-4806 (online)

Update to: Advancing butterfly systematics through genomic analysis

Jing Zhang[1,2,3], **Qian Cong**[1,3], **Jinhui Shen**[1,2], **Leina Song**[1,2], **and Nick V. Grishin**[1,2*]

Departments of [1]Biophysics, [2]Biochemistry, and [3]Eugene McDermott Center for Human Growth & Development,
University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9050, USA;
[*]Corresponding author: *grishin@chop.swmed.edu*

ABSTRACT. In this update, we propose (type species or type localities in parentheses): *Lochris* Grishin, **nom. nov.** (*Lasaia oileus* Godman, 1903; Lepidoptera: Riodinidae) as a new substitute name for *Locris* Grishin, 2025 preoccupied by *Locris* Stål, 1866 (*Cercopis rubra* Fabricius, 1794; Hemiptera: Cercopidae), 5 new species, and 5 new subspecies: *Emesis* (*Mandania*) *mandarina* Grishin, **sp. n.** (Brazil: Santa Catarina) and *Emesis* (*Mandania*) *mandela* Grishin, **sp. n.** (Venezuela, Carabobo) in Riodinidae Grote, 1895 (1827); others in Hesperiidae Latreille, 1809: *Telegonus* (*Rhabdoides*) *flavifimbro* Grishin, **sp. n.** (Colombia), *Urbanus* (*Urbanoides*) *dolus* Grishin, **sp. n.** (Colombia: Tolima), *Thespieus grandosul* Grishin, **sp. n.** (Brazil: Rio Grande do Sul); *Telegonus* (*Rhabdoides*) *alector obscuratus* Grishin, **ssp. n.** (Costa Rica: Puntarenas), *Burnsius communis tenebrunis* Grishin, **ssp. n.** (USA: OR, Klamath Co.), *Burnsius communis altus* Grishin, **ssp. n.** (USA: CA, Tuolumne Co.), *Ochlodes* (*Ochluma*) *sylvanoides paranapa* **ssp. n.** (USA: WY, Washakie Co.), and *Ochlodes* (*Ochluma*) *sylvanoides dempwolfi* **ssp. n.** (USA: ND, Slope Co.). Furthermore, we illustrate sequenced specimens, confirm the status of several species recently described based on limited material by sequencing additional specimens, and provide updated distribution data for these species.

Key words: taxonomy, classification, genomics, phylogeny, biodiversity.

ZooBank registration: http://zoobank.org/504B8C6D-D4AA-4489-8CE4-A636BC5F5426

Here, we provide updates and corrections to our recently published work (Zhang et al. 2025a), resulting from sequencing of additional specimens using the same methods within the same conceptual framework. Notations for DNA character states are: aly728.44.1:G672C meaning position 672 in exon 1 of gene 44 from scaffold 728 of the *Cecropterus lyciades* (Geyer, 1832) reference genome (prefix cne instead of aly is used for the *Calephelis nemesis* (W. H. Edwards, 1871) reference (Cong et al. 2017)) (Shen et al. 2017) is C, changed from G in the ancestor. When characters are given for the sister clade of the diagnosed taxon, we use: aly5294.20.2:A548A (not C), which means that position 548 in exon 2 of gene 20 on scaffold 5294 is occupied by the ancestral base pair A, which was changed to C in the sister clade (so it is not C in the diagnosed taxon). COI barcode positions follow the same format but lack a prefix ending in ':'. Complete exon sequences from the reference genome, with diagnostic positions for new taxa highlighted in green, are provided in the supplementary file < https://osf.io/fhrm6/ >. By linking to this file, we ensure that the characters used in diagnoses can be traced to their actual sequences.

Whole genome shotgun datasets we obtained and used in this study are available from the NCBI database < https://www.ncbi.nlm.nih.gov/ > under BioProject PRJNA1288563. Associated BioSample records include locality data and other collection information for all specimens sequenced by us and shown in the trees. Tree figures list the following information for each specimen, separated by "|": taxon name with comments in square brackets, DNA sample code, type status, general locality, and year of collection ("old" if not dated and likely collected 100–150 years ago). Type status abbreviations are: HT holotype, LT lectotype, ST syntype, NT neotype, PT paratype, AT allotype, PLT paralectotype; and if a synonym name is given (in parentheses, preceded by "=", and in addition by "‡" for unavailable names), type status refers to the synonym. Ultrafast bootstrap (Minh et al. 2013) values are shown at nodes. COI

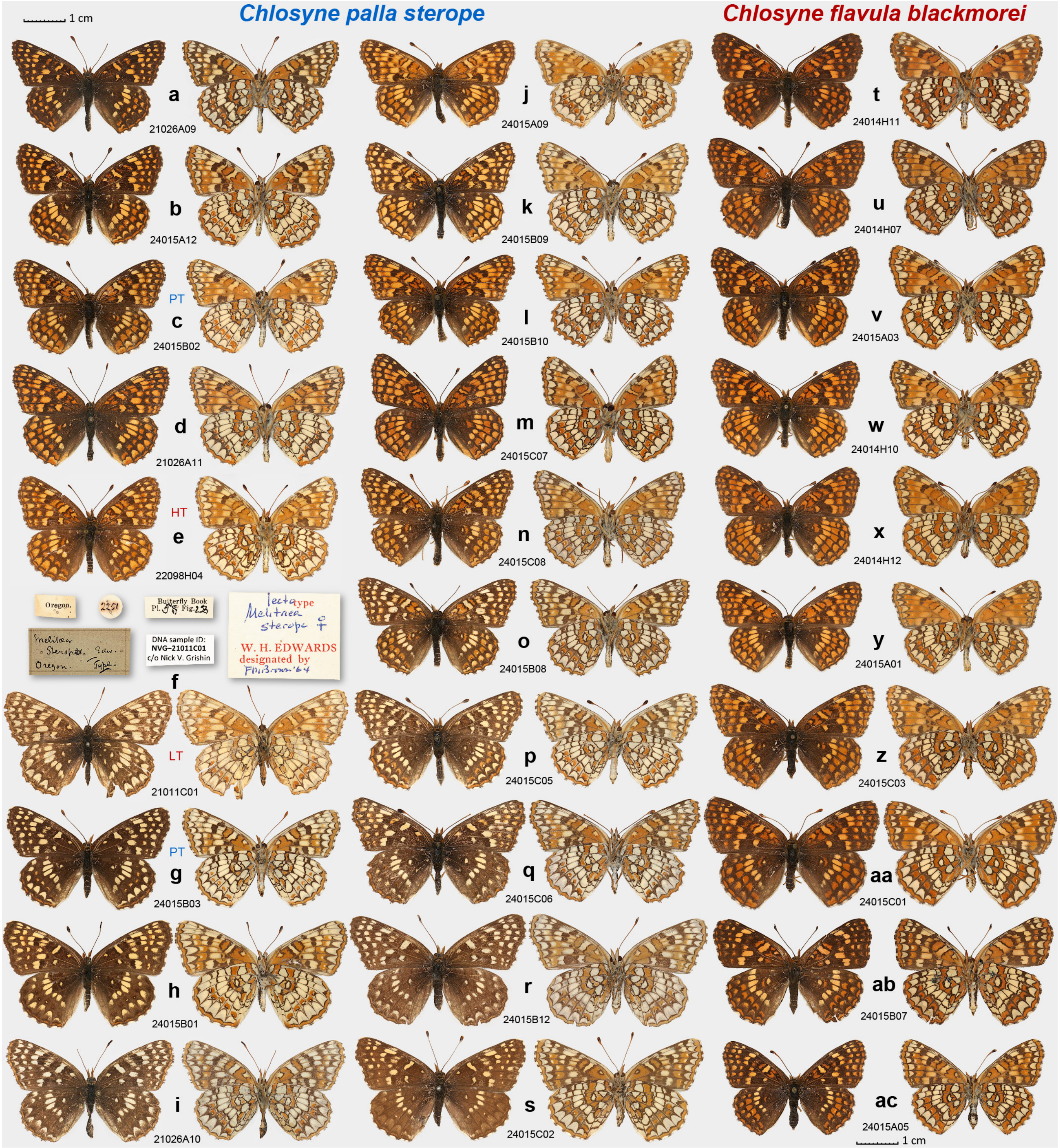


Fig. 1. Sequenced specimens of the *Chlosyne palla* group from Canada and USA in dorsal (left) and ventral (right) views: **a–s)** *C. palla sterope* from: **a–i)** the southern and **j–s)** the northern parts of the range and **t–ac)** *C. flavula blackmorei*: **a, d, i)** WA, Adams Co., 25 mi SW Ritzville [CSUC]; others in CNC unless indicated: **b, c, e–h)** OR, Wasco Co., Tygh Valley; **k–r)** WA, Okanogan Co.: **m)** Black Canyon, **o, p)** Pateros, others nr. Brewster; **j, s, t, v–z, aa, ac)** British Columbia: **j, s, w)** Osoyoos, **t)** Keremeos, **v)** Pavillon Lake, **x)** Clinton, **y)** Hedley, **z)** 100 Mile House, **aa)** Canim Lake, **ac)** Jesmond; **u)** Alberta, Waterton Lakes; **ab)** ID, Shoshone Co., Wallace. DNA sample numbers are: **a)** ♂ NVG-21026A09, **b)** ♂ NVG-24015A12, **c)** paratype ♂ of *Melitaea hewesi* Leussler, 1931 (junior subjective synonym of *C. palla sterope*) NVG-24015B02, **d)** ♂ NVG-21026A11, **e)** holotype ♂ of *M. hewesi* NVG-22098H04 [CAS] (no labels shown for this specimen), **f)** lectotype ♀ of *C. palla sterope* NVG-21011C01 [CMNH] (labels above), **g)** paratype ♀ *M. hewesi* NVG-24015B03, **h)** ♀ NVG-24015B01, **i)** ♀ NVG-21026A10, **j)** ♂ NVG-24015A09, **k)** ♂ NVG-24015B09, **l)** ♂ NVG-24015B10, **m)** ♂ NVG-24015C07, **n)** ♂ NVG-24015C08, **o)** ♂ NVG-24015B08, **p)** ♀ NVG-24015C05, **q)** ♀ NVG-24015C06, **r)** ♀ NVG-24015B12, **s)** ♀ NVG-24015C02, **t)** ♂ NVG-24014H11, **u)** ♂ NVG-24014H07, **v)** ♂ NVG-24015A03, **w)** ♂ NVG-24014H10, **x)** ♂ NVG-24014H12, **y)** ♂ NVG-24015A01, **z)** ♀ NVG-24015C03, **aa)** ♀ NVG-24015C01, **ab)** ♀ NVG-24015B07, **ac)** ♀ NVG-24015A05. Additional details in Zhang et al. (2025a).

barcode sequences reported here have been deposited in GenBank with accessions PV892283–PV892292. Abbreviations or acronyms for collections are listed in Acknowledgments.

Family Nymphalidae Rafinesque, 1815

Chlosyne palla sterope (W. H. Edwards, 1870) and *Chlosyne flavula blackmorei* Pelham, 2008: illustrations of selected sequenced specimens

Here, we compile photographs of several specimens of both sexes that were included in the phylogenetic tree published as fig. 2 in Zhang et al. (2025a) to illustrate phenotypic variation and interspecies differences in the northernmost populations of the two species: *Chlosyne palla* (Boisduval, 1852) (type locality in USA: California, Plumas Co.) and *Chlosyne flavula* (W. Barnes & McDunnough, 1918) (type locality USA: Colorado, Garfield Co., Glenwood Springs) (Zhang et al. 2023c, 2025a), specifically, *Chlosyne palla sterope* (W. H. Edwards, 1870) (type locality in USA: Oregon, Wasco Co.) and *Chlosyne flavula blackmorei* Pelham, 2008 (type locality Canada: British Columbia, Lytton) (Fig. 1). The two species are best differentiated by females: *C. palla sterope* is typically darker with cream-colored to nearly white spots and bands and usually a more elongated forewing apex; and *C. flavula blackmorei* has orange and orange-yellow spots and bands and a rounder forewing apex.

We were not able to find consistent wing pattern differences between the northern populations of *C. palla sterope* (Canada: British Columbia, Osoyoos and USA: Washington, Okanogan Co.) and its more southern populations (USA: Washington, Adams Co. and Oregon, Wasco Co.), with some specimens being rather similar (e.g., compare Fig. 1d with Fig. 1l or Fig. 1m for males, and Fig. 1g with Fig. 1q for females). Females are particularly alike. However, males from the northern populations are more variable, with some being more extensively orange (e.g., Fig. 1j) than typical *C. palla sterope*, or exhibiting cream-colored (rather than orange-yellow) discal bands (Fig. 1o). Genetically, all these specimens cluster together, forming a nuclear genome clade that also includes the lectotype of *C. palla sterope* (sequenced as NVG-21011C11 and used to assign this name to the clade), but partition into the northern and southern subclades; see fig. 2 in Zhang et al. (2025a).

Family Riodinidae Grote, 1895 (1827)

Lochris Grishin, nom. nov. is a new substitute name to replace *Locris* Grishin, 2025

http://zoobank.org/B13E52B7-663E-4C16-9ADD-D57302A19744

A subgenus of *Lasaia* H. Bates, 1868, *Locris* Grishin, 2025 (type species *Lasaia oileus* Godman, 1903) is a junior homonym of *Locris* Stål, 1866 (type species *Cercopis rubra* Fabricius, 1794), currently a valid genus of froghoppers (Hemiptera: Cercopidae) (Stål 1866). Here, according to Article 60.3 of the International Code of Zoological Nomenclature (1999), *Lochris* Grishin is proposed as a **new** substitute **name** that replaces *Locris* Grishin, 2025. According to the ICZN Code Article 67.8, the type species of *Lochris* Grishin, **nom. nov.** is *Lasaia oileus* Godman, 1903.

Emesis (*Mandania*) *mandarina* Grishin, new species

http://zoobank.org/98452F55-3EB2-4B5A-84B1-4D1C8FF074BE

(Figs. 2 part, 3)

Definition and diagnosis. Genomic analysis reveals that several specimens from Santa Catarina, Brazil, initially identified as *Emesis* (*Mandania*) *mandana* (Cramer, 1780) (type locality in Suriname) are genetically differentiated from it at the species level (Fig. 2); e.g., their COI barcodes differ by 0.9% (6 bp, barcodes do not differ strongly in this species group (Zhang et al. 2024, 2025b)), and, therefore, they represent a new species. This new species is similar to its sister *E. mandana* in having redder colors of the dorsal side in males, but differs from it by a more uniformly colored orange ventral side of the wings without more prominent redder and broader margins and the lack of a defined ventral hindwing spot at the tornus. Males (Fig. 3a, c) have smaller and more weakly expressed submarginal dark dots, better-separated dark markings in the postdiscal row on the ventral side, and typically darker (maroon-toned) background color of the dorsal side. Females (Fig. 3b) may have a rounder forewing with a more convex outer margin and a less prominently concave hindwing outer margin at the vein M_2, narrower dashes and crescents in the discal band on the dorsal side with a dash in cell M_2-M_3 being more strongly offset distad and aligned with the dash in cell M_3-CuA_1, and paler marginal areas on the ventral side. Due to its cryptic nature and unexplored individual variation, this species is best identified by DNA, with diagnostic base pairs in the nuclear genome: cne4739.1.2:C183T, cne339.14.2:A330T, cne339.14.2:C435T, cne37103.1.5:T462A, cne37103.1.5:T468C; and the COI barcode: T367C, A379C, T578C, T610C.

Barcode sequence of the holotype. Sample NVG-18044E12, GenBank PV892283, 658 base pairs:

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTTGGAACTTCACTAAGATTATTAATTCGAATAGAATTAGGAACTTCAGGATCATTAATTGGTGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCATTAATACTAGGAGCCCCAGATATAGCTTTTCCACGAA
TAAATAATATAAGATTTTGACTTTTACCTCCATCTTTAATTTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTGTACCCCCCACTTTCTTCTAATATTGC
TCACGGAGGTTCTTCCGTAGATTTAGCTATTTTTTCTTTACATTTAGCAGGAATTTCCTCAATTTTAGGTGCAATTAACTTTATTACTACTATTATTAATATACGAATTAATAATATATCA
TTTGATCAAATACCTTTATTTGTTTGATCTGTAGGAATTACAGCTCTTCTATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATTACTATACTATTAACAGATCGAAATTTAAATACAT
CATTCTTTGATCCTGCTGGTGGTGGTGATCCTATTTTATATCAACATTTATTT
```

Type material. Holotype: ♂ deposited in the National Museum of Natural History, Washington, DC, USA (USNM), illustrated in Fig. 3a, bears the following eight rectangular labels (1st handprinted, others

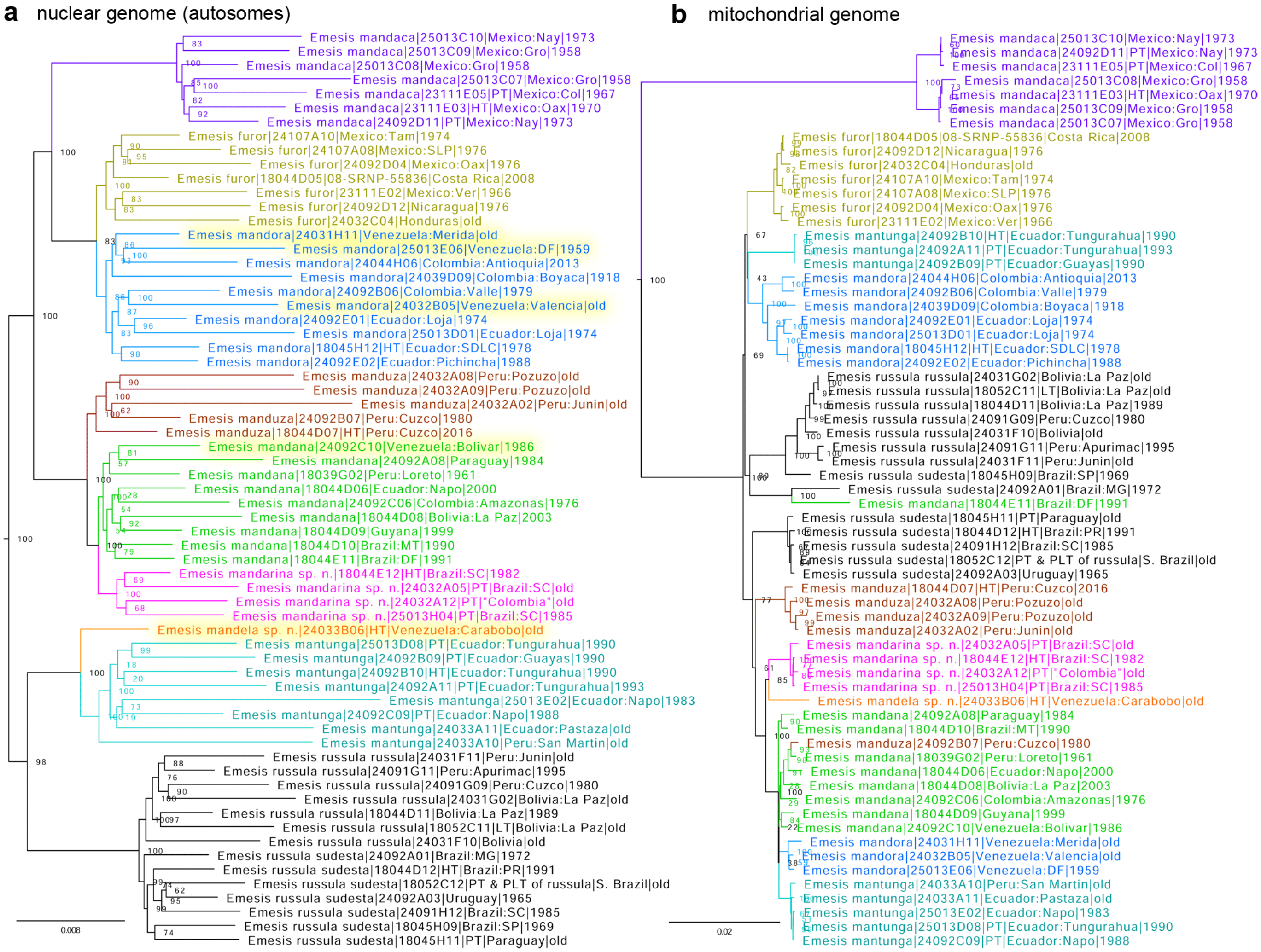


Fig. 2. Phylogenetic trees of *Emesis* (*Mandania*) species constructed from protein-coding regions in: **a)** the nuclear genome (autosomes), based on 826,947 positions, and **b)** the mitochondrial genome. Different species are colored differently: *E. mandaca* (violet), *E. furor* A. Butler and H. Druce, 1872 (olive), *E. mandora* (blue), *E. manduza* (brown), *E. mandana* (green), *E. mandarina* **sp. n.** (magenta), *E. mandela* **sp. n.** (orange), *E. mantunga* (cyan), and *E. russula* Stichel, 1910 (black). Labels of specimens from Venezuela are highlighted in yellow in (a).

Fig. 3. Type specimens of *Emesis* (*Mandania*) *mandarina* **sp. n.** from Brazil: Santa Catarina in dorsal (above or left of the panel letter) and ventral (below or right of the letter) views, data in text: **a)** holotype ♂ NVG-18044E12; **b)** paratype ♀ NVG-24032A05; **c)** paratype ♂ NVG-25013H04.

printed with handwritten text shown in italics; 4th blue, 5th yellow, the last red, others white): [Joinville | 18·IX·1982], [StaCatharina | Brazil], [Presented by | Robert E. Aronheim], [JHALL | -00*05*], [LEGS AWAY | FOR DNA], [DNA sample ID: | NVG-18044E12 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01466379], and [HOLOTYPE ♂ | Emesis (Mandania) | mandarina Grishin]. **Paratypes:** 1♂ and

2♀♀ from Brazil, Santa Catarina (last one likely mislabeled): 1♂ NVG-25013H04 Joinville, 4-Mar-1985, H. Miers leg. [MGCL] (Fig. 3c); 1♀ NVG-24032A05 Blumenau, old, coll. Staudinger [MFNB] (Fig. 3b); and 1♀ NVG-24032A12 "Colombia | R. Magdalena s", old, ex coll. H. Stichel, number 3280 [MFNB].

Type locality. Brazil: Santa Catarina, Joinville.

Etymology. The name is a fusion given to this relative of *mand*[ana from Santa Cat]*arina*, and is treated as a noun in apposition.

Distribution. Currently known only from Santa Catarina in Brazil.

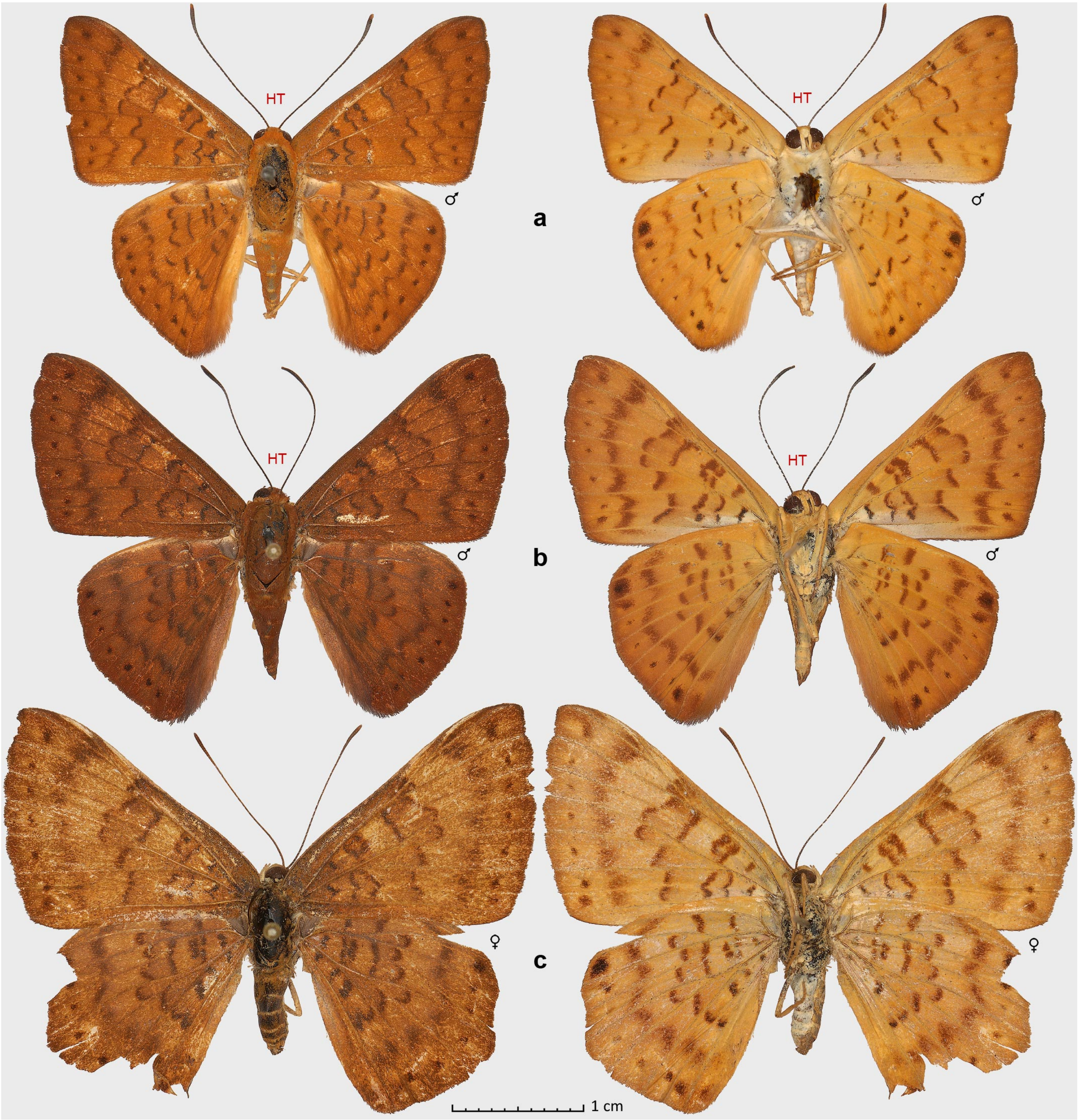


Fig. 4. Specimens of *Emesis* (*Mandania*) in dorsal (left) and ventral (right) views, data in text or below: **a)** *E. mandela* **sp. n.** holotype ♂ NVG-24033B06 from Venezuela and **b–c)** *E. mantunga* from Ecuador: **b)** holotype ♂ NVG-24092B10 Tungurahua, Topo, 18-20-Jun-1990, I. A. Villafuerte leg. [MGCL] and **c)** the first confirmed ♀ NVG-25013E02.

Emesis (*Mandania*) *mandela* Grishin, new species

http://zoobank.org/E971DCB7-6DA1-48D6-BF19-C1E866DE2226

(Figs. 2 part, 4a)

Definition and diagnosis. Genomic analysis reveals that a specimen from Venezuela (Fig. 4a) is sister to *Emesis* (*Mandania*) *mantunga* Grishin, 2025 (type locality in Ecuador: Tungurahua) (Fig. 4b), but is genetically differentiated at the species level (Fig. 2); e.g., their COI barcodes differ by 1.7% (11 bp, which is large for this species group (Zhang et al. 2024, 2025b)), and, therefore, it represents a new species. This new species differs from its relatives by males with paler wing color, which is rich, reddish-orange, similar in tone to burnt orange or rust. The ventral side of wings is yellower with a narrower and more weakly expressed postdiscal band of crescents but a crisper discal band; and the hindwing is more elongated towards the tornus, with a straighter outer margin. Due to its cryptic nature and unexplored individual variation, this species is best identified by DNA, with diagnostic base pairs in the nuclear genome: cne670.2.5:C84T, cne670.2.5:A102T, cne7425.1.3:A90G, cne7425.1.3:C111T, cne5229.9.3:T279A, cne22806.1.1:C183C (not T), cne22806.1.1:G184G (not A), cne22806.1.1:A198A (not T), cne5064.6.3:C75C (not T), cne5064.6.3:T80T (not A); and the COI barcode: T367C, T400C, A412G, T532C.

Barcode sequence of the holotype. Sample NVG-24033B06, GenBank PV892284, 658 base pairs:

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTTGGAACTTCACTAAGATTATTAATTCGAATAGAATTAGGAACTTCAGGATCATTAATTGGTGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCATTAATACTAGGAGCTCCAGATATAGCTTTTCCACGAA
TAAATAATATAAGATTTTGACTTTTACCTCCATCTTTAATTTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTGTACCCCCCACTTTCTTCTAATATTGC
TCACGGAGGTTCTTCCGTAGATTTAGCTATTTTTTCCTTACATTTAGCGGGAATTTCCTCAATTTTAGGTGCAATTAACTTTATTACTACTATTATTAATATACGAATTAATAATATATCA
TTTGATCAAATACCTTTATTTGTTTGATCTGTAGGAATTACAGCTCTCCTATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATTTAAATACAT
CATTCTTTGATCCTGCTGGTGGTGGTGATCCTATTTTATATCAACATTTATTT
```

Type material. Holotype: ♂ deposited in the Museum für Naturkunde, Berlin, Germany (MFNB), illustrated in Fig. 4a, bears the following five printed rectangular labels (text in italics handwritten), four white: [P$^{\underline{to}}$ Cabello | Hahnel], [Coll. | Staudinger], [DNA sample ID: | NVG-24033B06 | c/o Nick V. Grishin], [{QR Code} MfN URI | http://coll.mfn- | berlin.de/u/ | 09f2f9], and one red [HOLOTYPE ♂ | Emesis (Mandania) | mandela Grishin].

Type locality. Venezuela: Carabobo, Puerto Cabello.

Etymology. The name is a fusion given to this relative of *mand*[ana from Venezu]*ela*, and is treated as a noun in apposition.

Distribution. Currently known only from the holotype collected in coastal Venezuela.

Comment. This new species is the third *Emesis* (*Mandania*) species we recorded in Venezuela, in addition to *Emesis* (*Mandania*) *mandana* (Cramer, 1780) (type locality in Suriname) and *Emesis* (*Mandania*) *mandora* Grishin, 2024 (type locality in Ecuador: Santo Domingo) (Fig. 2 yellow highlight).

Additional records of *Emesis* (*Mandania*) *mantunga* Grishin, 2025

Through expanded genomic sequencing (Fig. 2), we found three more specimens of *Emesis* (*Mandania*) *mantunga* Grishin, 2025 (type locality Ecuador: Tungurahua Province, Topo; originally described from five males), including the first confirmed female (NVG-25013E02, Ecuador: Napo Province, Puerto Misahuallí, 6-Nov-1983, D. & J. Jenkins [MGCL], Fig. 4c), an additional male from eastern Ecuador (NVG-24033A11, Pastaza Province, Sarayacu, old, R. Haensch S., Stichel collection number 3282 [MFNB]), and extend the distribution of this species to the eastern slopes of the Andes in northern Peru (♂ NVG-24033A10, San Martín Department, Tarapoto, old, Stichel collection number 4338 [MFNB]).

Family Hesperiidae Latreille, 1809

Telegonus (*Rhabdoides*) *alector obscuratus* Grishin, new subspecies

http://zoobank.org/C615B4FA-4DD4-491D-B7EB-0C54C481D1DF

(Figs. 5 part, 6a)

Definition and diagnosis. Genomic analysis reveals that a male from Costa Rica initially identified as *Telegonus* (*Rhabdoides*) *gilberti* (Freeman, 1969) (type locality in Mexico: San Luis Potosí) is genetically

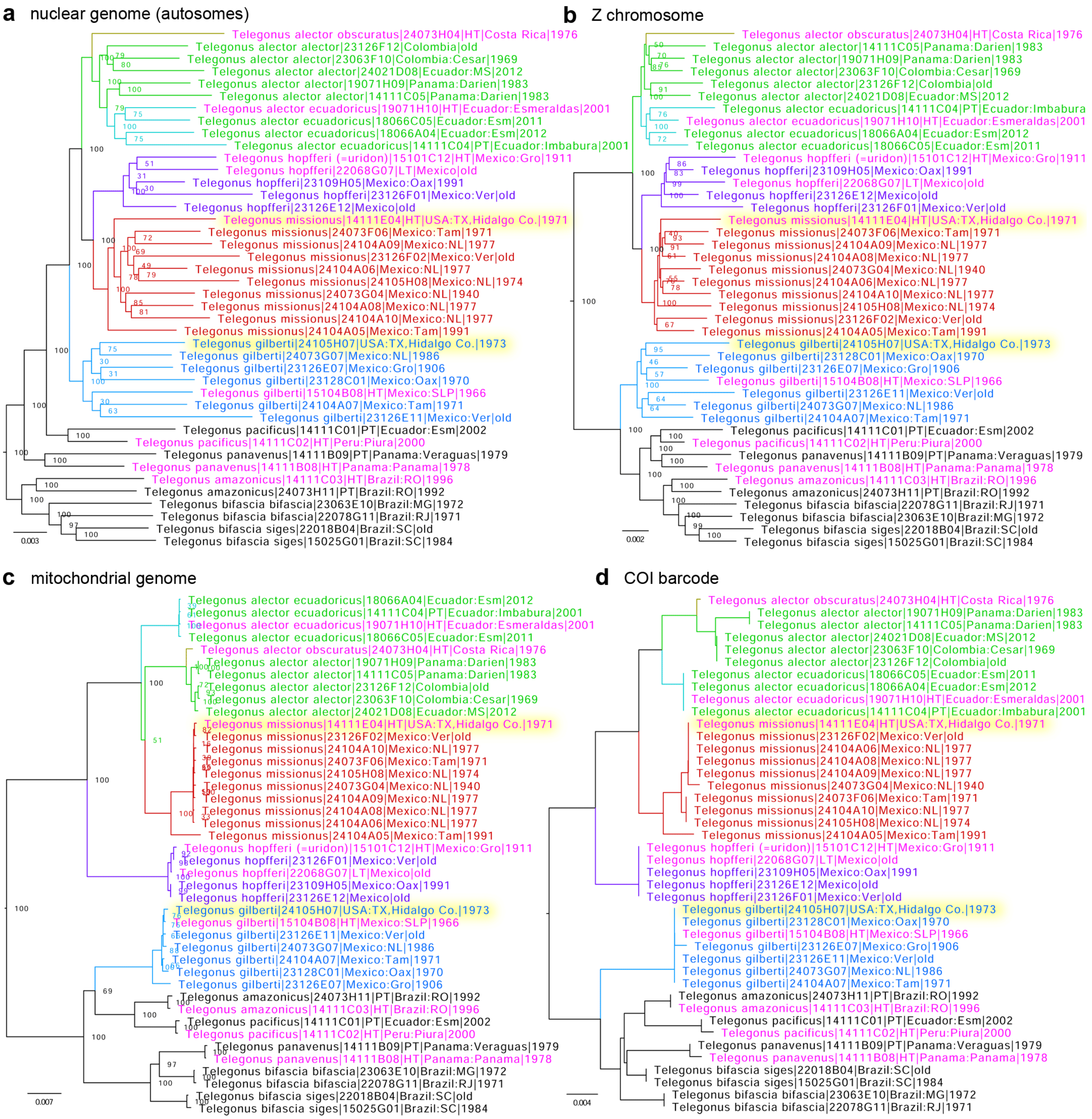


Fig. 5. Phylogenetic trees of *Telegonus* (*Rhabdoides*) species from the *alector* group constructed from protein-coding regions in: **a)** the nuclear genome (autosomes), based on 7,793,130 positions, **b)** the Z chromosome, based on 187,920 positions, **c)** the mitochondrial genome, and **d)** a BioNJ (Gascuel 1997) dendrogram constructed from COI barcodes using the phylogeny.fr server (Dereeper et al. 2008). Different taxa are colored differently: *T. alector* (green, with the *T. alector obscuratus* **ssp. n.** branch in olive and the *T. alector ecuadoricus* clade in cyan), *T. hopfferi* (purple), *T. missionus* (red), and *T. gilberti* (blue). Primary type specimens are labeled in magenta and specimens from the U.S. are highlighted in yellow.

differentiated from it at the species level (Fig. 5); e.g., their COI barcodes differ by 3.3% (22 bp). Instead, according to genomic trees, this male is most closely related to *Telegonus* (*Rhabdoides*) *alector* (C. Felder & R. Felder, 1867) (type locality in Colombia: Bogotá) while phenotypically differing from it by the lack of a white smudge on the dorsal forewing and smaller size (Fig. 6a). This male is not prominently different genetically from the nominate subspecies of *T. alector* (Fig. 6b). Therefore, due to phenotypic differences from it and *Telegonus* (*Rhabdoides*) *alector ecuadoricus* Grishin, 2025 (type locality in

Ecuador: Esmeraldas) (Fig. 6c), this male represents a new subspecies. This new subspecies keys (incompletely) to "*Astraptes alector alector*" C.14.26(b) in Evans (1952), having blue rather than green wing bases, but males lack the white smudge in the discal area of the dorsal forewing. The new subspecies differs from other relatives by the following combination of characters in males: the dorsal side of wings is dark-brown with brilliant-blue (not green) bases and only a very weak trace (absent in *T. gilberti*) of the discal forewing pale smudge in the cell CuA$_1$-CuA$_2$; the ventral side of the wings is brown; the ventral forewing has a discal white band from within the discal cell widening to the vein 1A+2A and ending in a

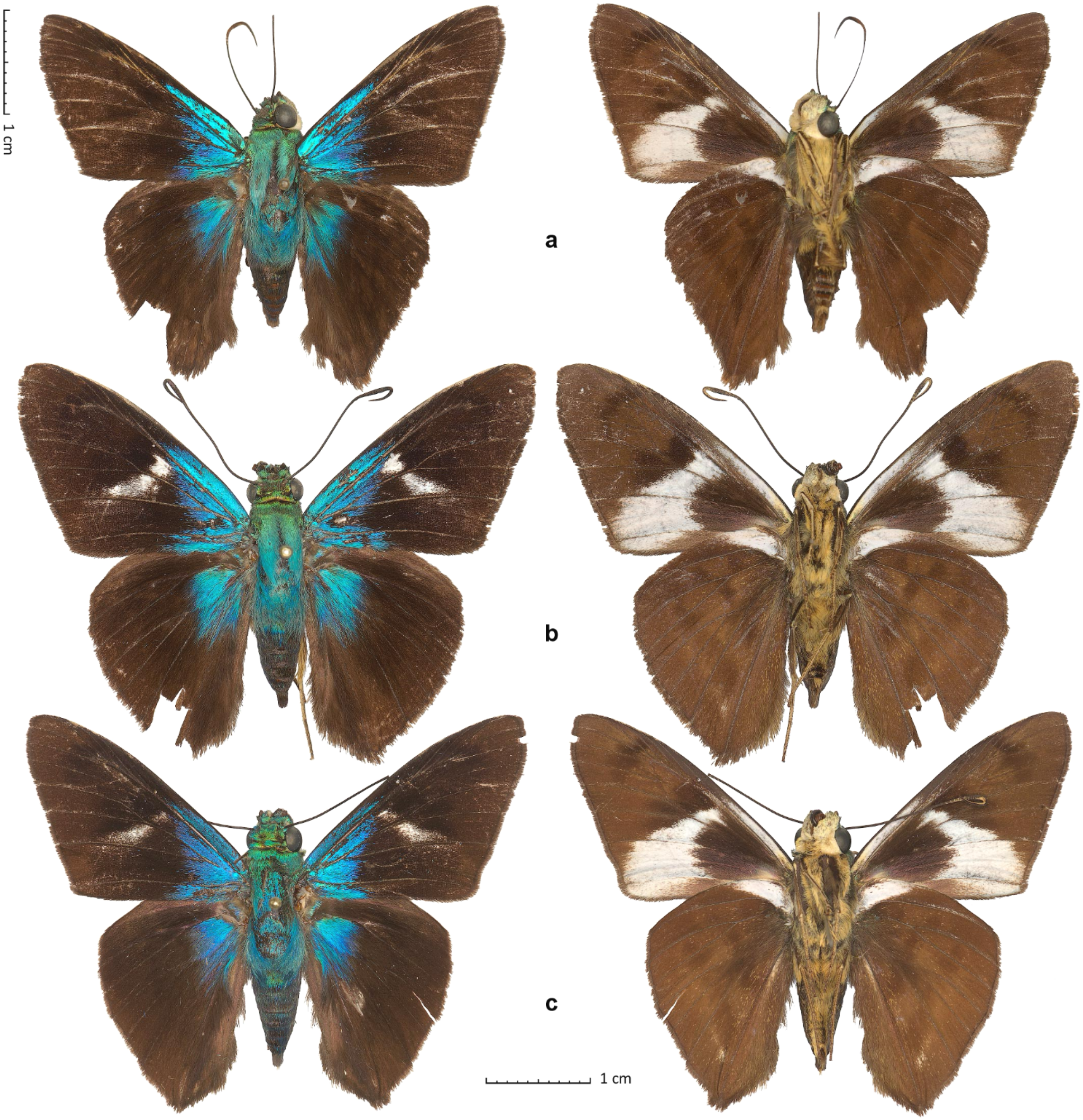


Fig. 6. Subspecies of *Telegonus* (*Rhabdoides*) *alector* in dorsal (left) and ventral (right) views, data in text of below: **a)** *T.* (*R.*) *alector obscuratus* **ssp. n.** holotype ♂ NVG-24073H04 from Costa Rica; **b)** *T.* (*R.*) *alector alector* ♂ NVG-24021D08 from Ecuador: Morona-Santiago, San Isidro, Macas, 1250 m, GPS −02.12, −78.10, 19-Oct-2012, J.-C. Petit leg. [SMF]; and **c)** *T.* (*R.*) *alector ecuadoricus* holotype ♂ NVG-19071H10 from Ecuador: Esmeraldas, Río Chuchuví, km 12.5 of Lita–San Lorenzo Road, 800–900 m, GPS 0.8835, −78.5150, Mar-2001, I. Aldas leg. [USNM].

smudge near the tornus, and a prominent white costal area from the base to about a quarter of the wing length, somewhat overscaled orange-yellow at the very base; the ventral hindwing has a white triangular area by the costal margin (reaching to about its third) at the base, slightly overscaled with brown at the distal angle, but otherwise with a straight and sharply defined posterior edge; and orange-yellow body beneath. Due to its cryptic nature and unexplored individual variation, this subspecies is best identified by DNA, with diagnostic base pairs in the nuclear genome: aly259.26.2:A1801C, aly2250.22.2:A36C, aly1084.16.5:C126T, aly2370.12.8:T36C, aly1531.17.2:A310C, aly1158.5.1:A351A (not G), aly1315.19.5:G78G (not C), aly1315.19.5:G81G (not C), aly1315.19.5:A90A (not C), aly322.10.7:T48T (not C). In the COI barcode, the new subspecies is not confidently different from the nominate subspecies.

Barcode sequence of the holotype. Sample NVG-24073H04, GenBank PV892285, 658 base pairs:

```
AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAATTGGTACTTCTTTAAGATTACTTATTCGAACTGAATTAGGAACTCCTGGATCTTTAATTGGAGATGATCAAATTTACAATACT
ATTGTAACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAATTCCATTAATAATAGGAGCCCCTGATATAGCTTTCCCCCGAA
TAAATAATATAAGATTTTGACTTTTACCCCCATCATTAACTTTATTAATTTCAAGAAGAATTGTAGAAAATGGTGCTGGAACAGGATGAACAGTTTATCCCCCTCTTTCATCTAATATTGC
CCATCAAGGAGCATCAGTTGACTTAGCAATTTTCTCTTTACATTTAGCTGGTATTTCTTCTATTCTTGGAGCTATTAATTTTATCACAACAATTATTAATATACGAATTAATAGCCTATCT
TTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATCACAGCATTATTATTATTACTTTCTTTACCAGTTTTAGCAGGAGCCATTACTATATTATTAACTGATCGAAATTTAAATACTT
CATTTTTTGATCCAGCTGGAGGAGGAGATCCAATTTTATATCAACACTTATTT
```

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 6a, bears the following four rectangular labels (1st handwritten, others printed with handwritten text shown in italics), three white: [C.RICA: PUNTARENAS | Corcovado; 20.ix.1976 | P. DeVries], [Allyn Museum | Acc. 19*78-20*], [DNA sample ID: | NVG-24073H04 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Telegonus (Rhabdoides) alector | obscuratus Grishin].

Type locality. Costa Rica: Puntarenas Province, Corcovado National Park.

Etymology. In Latin, *obscuratus* means darkened, made dark, or having become dark, and is given for the lack of the white smudge in the middle of the forewing in males characteristic of other *T. alector* subspecies. The name is a perfect passive participle.

Distribution. Currently known only from the holotype collected in southern Costa Rica.

Additional specimens of *Telegonus* (*Rhabdoides*) *missionus* Grishin, 2025 confirm it as a species-level taxon

Genomic sequencing of additional specimens of *Telegonus* Hübner, [1819] (type species *Papilio talus* Cramer, 1777) reveals that *Telegonus* (*Rhabdoides*) *missionus* Grishin, 2025 (type locality USA: Texas, Hidalgo Co., Mission, holotype sequenced as NVG-14111E04) is a species widely distributed in eastern Mexico, recorded from the states of Tamaulipas, Nuevo Leon, and Veracruz (Fig. 5 red), thus confirming it as a species-level taxon, and not an unusual single specimen. This recently described species (Zhang et al. 2025a) is sympatric with *Telegonus* (*Rhabdoides*) *gilberti* H. Freeman, 1969 (type locality in Mexico, San Luis Potosí, holotype sequenced as NVG-15104B08) over its range, with sequenced specimens of the latter species from the same three Mexican states shown in the trees (Fig. 5 blue). Both species (*T. missionus* and *T. gilberti*) have been recorded from Hidalgo County in Texas, USA (Fig. 5 highlighted yellow). In the nuclear genome trees, *T. missionus* is sister to *Telegonus* (*Rhabdoides*) *hopfferi* (Plötz, 1881) (type locality in Mexico, probably Oaxaca), a southern Mexico species, and specimens of both species were sequenced from Veracruz, (Fig. 5a, b).

As in our previous study (Zhang et al. 2025a), we observe confidently supported incongruence between the three phylogenetic trees of the *T. alector* species group (Fig. 5). In the Z chromosome tree (Fig 5b) and the mitochondrial genome tree (Fig 5c), the *T. alector* group partitions into two prominently separated clades with *T. gilberti* being in the second clade, thus more strongly differentiated genetically from the three species in the first clade: *Telegonus* (*Rhabdoides*) *alector* (C. Felder & R. Felder, 1867) (type locality in Colombia), *T. hopfferi*, and *T. missionus*; while in the tree constructed from protein-coding regions of autosomes (Fig 5a), all four species belong to the same clade, with *T. gilberti* being sister to the other three species. In addition to males, genomic sequencing also reveals females of *T. missionus*, and one is illustrated here for the first time (Fig. 7). Females are similar to males in their

Fig. 7. A female of *Telegonus* (*Rhabdoides*) *missionus* sequenced as NVG-24073G04, Mexico: Nuevo Leon, Villa Santiago, Hacienda Vista Hermosa,1500 ft, 17-Jun-1940, Hoogstraal & Knight leg. [MGCL].

darker appearance compared to females of related species (but paler than a typical *T. missionus* male), with a reduced white band on the ventral forewing, which has a beige (i.e., white scales sprinkled over brown) costal area from the base to about a third of the forewing length; a white triangle partly overscaled with brown at the base of the ventral hindwing; weakly expressed but noticeable pale overscaling in the discal area of the dorsal forewing corresponding to the white ventral band; and darker (not prominently orange-yellow) ventral side of the body.

Telegonus (*Rhabdoides*) *flavifimbro* Grishin, new species

http://zoobank.org/16CBDDCE-329A-402A-9C25-25D6790F53EC

(Figs. 8 part, 9b–c)

Definition and diagnosis. Genomic analysis reveals that two females from Colombia, initially identified as *Telegonus* (*Rhabdoides*) *chiriquensis* Staudinger, 1875 (type locality in Panama: Chiriquí) are genetically differentiated from it at the species level (Fig. 8); e.g., their COI barcodes differ by 4.1% (27

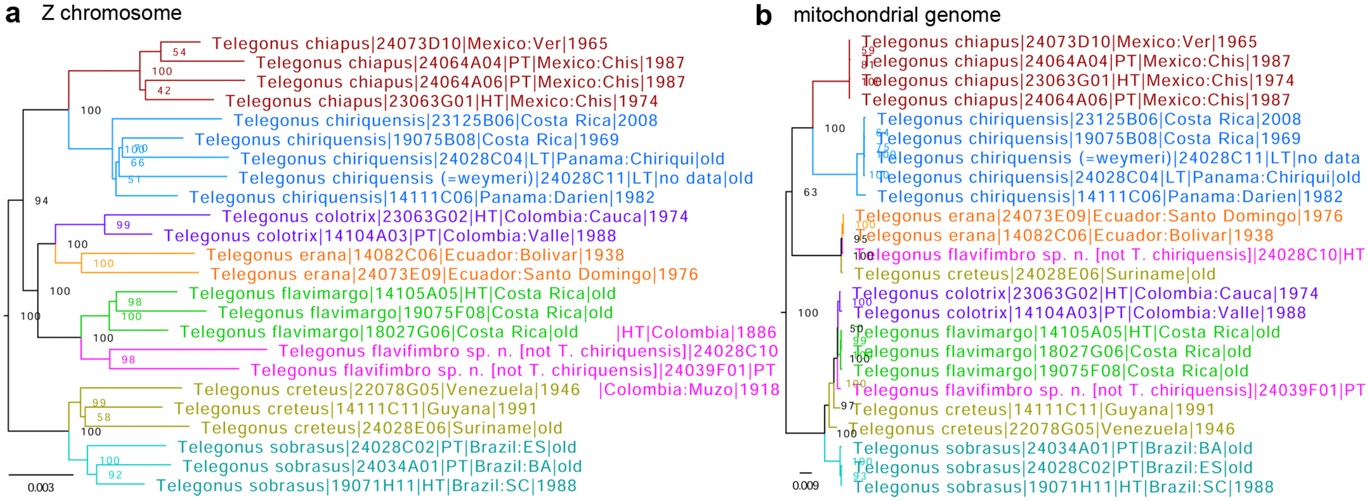


Fig. 8. Phylogenetic trees of selected *Telegonus* (*Rhabdoides*) species from the *creteus* group constructed from protein-coding regions in: **a)** the Z chromosome, based on 240,963 positions, and **b)** the mitochondrial genome. Different species are colored differently: *T. chiapus* (maroon), *T. chiriquensis* (blue), *T. colotrix* (purple), *T. erana* (orange), *T. flavimargo* (green), *T. flavifimbro* **sp. n.** (magenta; data lines are folded above (HT) and below (PT) to fit the page), *T. creteus* (olive), and *T. sobrasus* (cyan).

bp). Instead, these females form a nuclear genome clade sister to *Telegonus* (*Rhabdoides*) *flavimargo* Grishin, 2025 (type locality in Costa Rica), also differing from it at the species level, e.g., COI barcodes of the holotypes differ by 4.0% (26 bp), and, therefore, they represent a new species. This new species keys to "*Astraptes chiriquensis chiriquensis*" C.14.30(a) in Evans (1952), but differs from it and other relatives by the following combination of characters in females: an iridescent area at the base of the dorsal forewing is similar to or more developed than in *T. chiriquensis* but less extensive and greener than in *T. flavimargo*; the tornal area of the ventral forewing is even darker than in *T. flavimargo*; a yellow submarginal region on the ventral hindwing is the broadest close to the middle of the wing and narrowing towards the tornus, slightly broader than in *T. flavimargo*; a dark postdiscal band on the ventral forewing that is equidistant from the apical and discal bands (not closer to the apical band); more strongly expressed dark bands on the dorsal forewing; and more saturated in color and brighter orange-yellow fringes on the hindwing. Due to its cryptic nature and unexplored individual variation, this species is best

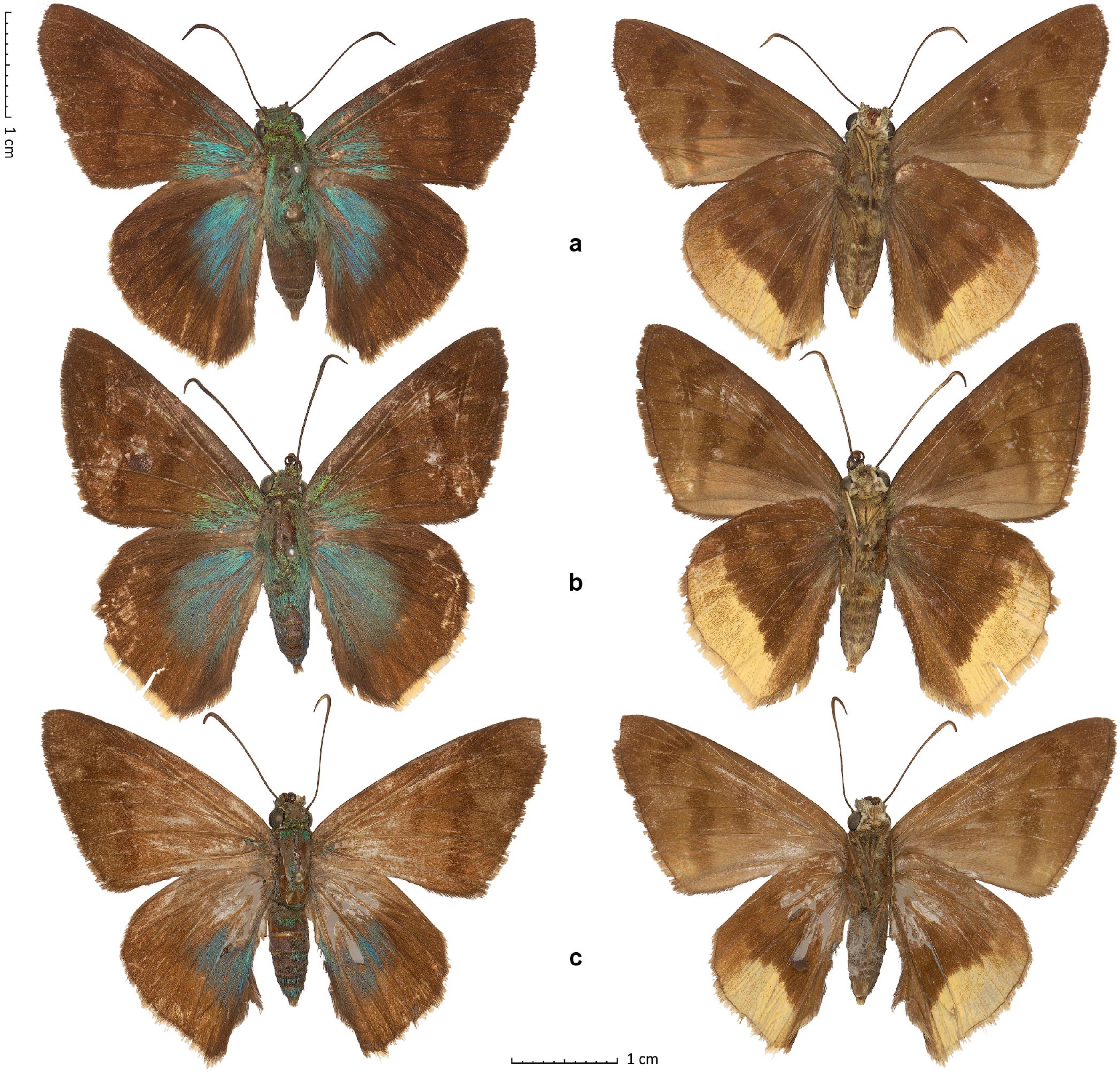


Fig. 9. Type specimens of *Telegonus* (*Rhabdoides*) in dorsal (left) and ventral (right) views, data below or in text: **a)** *T.* (*R.*) *flavimargo* holotype ♀ NVG-14105A05 from Costa Rica: Limón Province, Guapiles, elevation 850' and **b–c)** *T.* (*R.*) *flavifimbro* **sp. n.** ♀♀ from Colombia: **b)** holotype NVG-24028C10 and **c)** paratype NVG-24039F01.

identified by DNA, with diagnostic base pairs in the nuclear genome: aly2487.3.2:C51T, aly2487.3.2:C52T, aly275209.7.3:G198A, aly3614.1.6:C40T, aly393.1.23:C57G. In the COI barcode, this new species may not differ from others due to mitochondrial introgression among its relatives.

Barcode sequence of the holotype. Sample NVG-24028C10, GenBank PV892286, 658 base pairs:

```
AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAATCGGAACTTCTTTAAGATTACTTATTCGAACTGAATTAGGAACCCCAGGATCTTTAATTGGAGACGATCAAATTTATAACACT
ATTGTAACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTCCCATTAATAATAGGAGCTCCTGATATAGCTTTTCCTCGTA
TAAATAATATAAGATTTTGACTTCTACCCCCATCATTAACTTTATTAATTTCAAGAAGAATTGTTGAAAATGGTGCTGGAACAGGATGAACAGTTTATCCCCCTCTTTCATCTAATATTGC
CCACCAAGGAGCATCAGTTGATTTAGCTATTTTTTCCCTACATTTAGCTGGTATTTCTTCTATTTTAGGAGCTATTAATTTTATTACAACAATTATTAACATAAAAATTAATAATTTATCT
TTTGATCAAATACCTTTATTTGTATGAGCTGTTGGAATTACAGCATTATTATTATTACTTTCATTACCAGTTTTAGCAGGAGCTATTACTATATTATTAACTGATCGAAATTTAAATACTT
CATTTTTTGATCCAGCAGGAGGAGGAGACCCAATTTTATACCAACATTTATTT
```

Type material. Holotype: ♀ deposited in the Museum für Naturkunde, Berlin, Germany (MFNB), illustrated in Fig. 9b, bears the following seven rectangular labels (first four handwritten, others printed), six white: [Columbia | 86 Klbr.], [201.], [T. chiriquensis | ♀ var.], [chiriquensis | var.], [{QR Code} MfN URI | http://coll.mfn- | berlin.de/u/ | 09ec52], [DNA sample ID: | NVG-24028C10 | c/o Nick V. Grishin], and one red [HOLOTYPE ♀ | Telegonus (Rhabdoides) | flavifimbro Grishin]. **Paratype:** 1♀: NVG-24039F01 Colombia, Boyacá, Muzo, Mar-1918, W. Gerstner leg. [SMNS] (Fig. 9c).

Type locality. Colombia, possibly in the eastern Andes.

Etymology. Formed similarly to *flavimargo*, the name is given for the orange-yellow fringes (*fimbia* in Latin), particularly noticeable in the holotype of this species. The name is also longer to indicate a more southern distribution of this species and is treated as a noun in apposition.

Distribution. Currently known only from Colombia.

Telegonus (*Rhabdoides*) *adoba* (Evans, 1952), stat. nov. is a species distinct from *Telegonus* (*Rhabdoides*) *cretatus* Hayward, 1939

Genomic analysis of over two dozen *Telegonus* (*Rhabdoides*) *cretatus* Hayward, 1939 (type locality in Ecuador: Napo) specimens from across the range reveals that they partition into two comb-like clades genetically differentiated at the species level (Fig. 10). The first clade includes specimens from French Guiana, Venezuela, Ecuador, Peru, Bolivia, and Amazonian Brazil and corresponds to the nominate subspecies. The second clade is composed of specimens from the Atlantic states of Brazil from Bahia to Santa Catarina and represents a taxon originally described as a subspecies *Astraptes cretatus adoba* Evans, 1952 (type locality in Brazil: Espírito Santo) and currently treated as such, but now placed in *Telegonus* (*Rhabdoides*). Although it differs by only 0.6% (4 bp) from the nominate subspecies, this difference is consistent throughout the range, and the nuclear genome clades suggest a species-level distinction. Phenotypically, the Atlantic taxon is darker than the Amazonian (e.g., nearly lacking the ventral forewing green/white area by the costal margin at the base) and has a typically less robust serrated dorsal ridge of the harpe with a more rounded ventrocaudal angle. Therefore, we propose *Telegonus* (*Rhabdoides*) *adoba* (Evans, 1952), **stat. nov.** is a species distinct from *Telegonus* (*Rhabdoides*) *cretatus* Hayward, 1939.

Telegonus (*Rhabdoides*) *flavimargo* Grishin, 2025, *Telegonus* (*Rhabdoides*) *panamus* Grishin, 2025, and *Telegonus* (*Rhabdoides*) *tatus* Grishin, 2025 are confirmed as species-level taxa by sequencing of additional specimens

Genomic analysis enables us to confidently propose new species from a single specimen of either sex that is genetically differentiated from others at the species level (Zhang et al. 2025a). However, even fortified with the whole genome shotgun dataset, this single-specimen approach carries a risk of describing a hybrid or a contaminated dataset as a "species," despite all the precautions and careful analysis we undertake. Therefore, we strive to find and sequence additional specimens of the newly proposed species and investigate them further. Here, we confirm the species-level status of *Telegonus* (*Rhabdoides*) *flavimargo* Grishin, 2025 (type locality in Costa Rica: Limón, 2 additional specimens) (Fig. 8 green) and

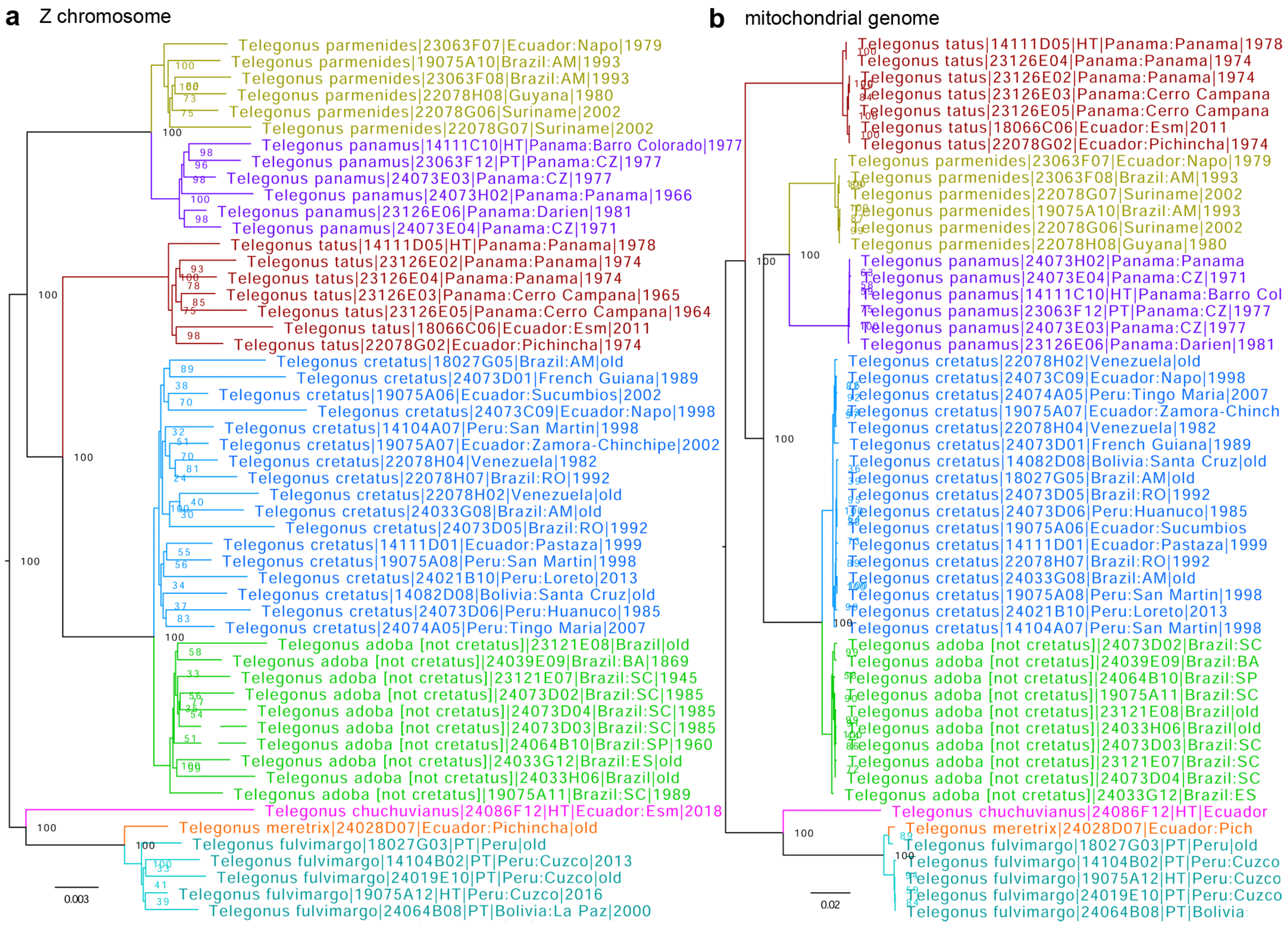


Fig. 10. Phylogenetic trees of *Telegonus* (*Rhabdoides*) species from the *parmenides* group constructed from protein-coding regions in: **a)** the Z chromosome, based on 267,339 positions, and **b)** the mitochondrial genome. Different species are colored differently: *T. parmenides* (olive), *T. panamus* (purple), *T. tatus* (maroon), *T. cretatus* (blue), *T. adoba* **stat. nov.** (green), *T. chuchuvianus* (magenta), *T. meretrix* (orange), and *T. fulvimargo* (cyan). Gaps in terminal branches indicate that a segment of a branch was cut out to reduce its length (to allow an increase in the font size), i.e., a branch with a gap is longer than shown.

Telegonus (*Rhabdoides*) *tatus* Grishin, 2005 (type locality in Panama: Panamá, 6 additional specimens) (Fig. 10 maroon) proposed from a single specimen and *Telegonus* (*Rhabdoides*) *panamus* Grishin, 2025 (type locality in Panama: Barro Colorado Island, 4 additional specimens) originally described from the holotype and the paratype (Fig. 10 purple). For all these species, additional specimens group closely with the holotypes in the genomic trees, resulting in comb-like clades characteristic of conspecific specimens. Although for *T. flavimargo* all three known specimens are from the same locality (Costa Rica: Limón Province, Guapiles), we are able to extend the known distribution for the other two species: *T. panamus* has been recorded from both central (around the Panama Canal) and eastern Panama (Darien) (Fig. 10 purple); and two specimens from northern Ecuador fall in the same clade among *T. tatus* specimens from central Panama and therefore, we identify them as this species (Fig. 10 maroon).

A modified preliminary taxonomic list of *Telegonus* (*Rhabdoides* Scudder, 1889) species from the clade analyzed in Zhang et al. (2025) and this work

Additional genomic sequencing and analyses suggest several revisions to our preliminary taxonomic list of species from the subgenus *Rhabdoides* Scudder, 1889 (only from the clade we have analyzed). The list from Zhang et al. (2025a) is updated below, using the same rationale and format. We introduced a new species and a new subspecies and revised the status of one subspecies to species. Furthermore, tweaks to the order were made. For instance, *Telegonus chuchuvianus* Grishin, 2025 was moved next to its most likely sister group of two species: *Telegonus meretrix* (Hewitson, 1876) and *Telegonus fulvimargo* Grishin, 2025, as evidenced by strong statistical support in both the Z chromosome and the mitochondrial genome trees (Fig. 10). The former species was placed before the latter two, because it lacks the yellower ventral hindwing margin characteristic of the latter, and the next species group in the list (the *latimargo* species group) starts with pale-margined species.

In the following arrangement from Zhang et al. (2025a), refined below, species of *Rhabdoides* excluding the clades with *Telegonus anaphus* (Cramer, 1777) and *Telegonus cellus* (Boisduval & Le Conte, [1837] are given. The list also includes species discovered by Steinhauser (1987) ("four new species will be added to the group") that fall within these species groups but remain unpublished, shown in gray font. Type localities (general area only: state, region, department, or county) are in gray font. New taxa described in this study and the category of taxonomic change are in red font. Taxonomic treatment before this work (for valid names) and comments are given in smaller font following a vertical bar | after the type locality; an equal sign = precedes synonyms given in their original genus combination; and a double dagger ‡ marks unavailable names. The list covers 48 valid taxa and 4 yet undescribed species, comprising 46 species (1 newly proposed here and 1 formerly treated as a subspecies) and 6 additional subspecies (1 new).

Genus ***Telegonus*** Hübner, [1819]; type species *Papilio talus* Cramer, 1777

Subgenus *Rhabdoides* Scudder, 1889; type species *Eudamus cellus* Boisduval & Le Conte, [1837]

alector species group

Telegonus alector (C. Felder & R. Felder, 1867)
- *Telegonus alector obscuratus* Grishin, **ssp. n.**; Costa Rica: Puntarenas
- *Telegonus alector alector* (C. Felder & R. Felder, 1867); Colombia: Bogotá
- *Telegonus alector ecuadoricus* Grishin, 2025; Ecuador: Esmeraldas

Telegonus hopfferi (Plötz, 1881); Mexico [likely C or S Mexico]
- = *Thracides uridon* Dyar, 1912; Mexico: Guerrero

Telegonus missionus Grishin, 2025; USA: Texas, Hidalgo Co.
Telegonus gilberti (Freeman, 1969); Mexico: San Luis Potosí
Telegonus panavenus Grishin, 2025; Panama: Panamá
Telegonus pacificus Grishin, 2025; Peru: Piura
Telegonus amazonicus Grishin, 2025; Brazil: Rondônia
Telegonus bifascia (Herrich-Schäffer, 1869)
- *Telegonus bifascia bifascia* (Herrich-Schäffer, 1869); [likely Brazil]
- *Telegonus bifascia siges* Mabille, 1903; Brazil [likely S]

elorus species group

Telegonus crana (Evans, 1952); Guatemala: San Gerónimo
- = *Astraptes escalantei* Freeman, 1967; Mexico: Chiapas | junior subjective synonym

Telegonus pallidus Grishin, 2025; Panama: Darién
Telegonus sp. undescribed #1, Steinhauser MS.; S. America
Telegonus subfuscus Grishin, 2025; Brazil: Santa Catarina
Telegonus cyprus (Evans, 1952)
- *Telegonus cyprus crilla* (Evans, 1952); Ecuador: Zamora
- *Telegonus cyprus cyprus* (Evans, 1952); Bolivia: Yungas & La Paz

Telegonus elorus (Hewitson, 1867); no data [likely SE or S Brazil]
- = *Eudamus blasius* Plötz, 1881; "Cuba" [likely SE or S Brazil] | junior subjective synonym
- = *Telegonus pheres* Mabille, 1903; Brazil: Santa Catarina | junior subjective synonym
- = *Telegonus subblasius* Strand, 1921; Argentina: Misiones | junior subjective synonym

Telegonus elorianus Grishin, 2025; unknown, likely SE or S Brazil *creteus* species group

- ***Telegonus grullus*** (Mabille, 1888); Panama: Chiriquí
- ***Telegonus*** sp. undescribed #2, Steinhauser MS.; Brazil
- ***Telegonus perumazon*** Grishin, 2025; Peru: Madre de Dios
- ***Telegonus steinhauseri*** Grishin, 2025; Mexico: Veracruz
    - =‡ *Telegonus chiriquensis* form *godmani* Williams, 1927; Mexico (Tab) and Nicaragua | infrasubspecific
- ***Telegonus chiapus*** Grishin, 2025; Mexico: Chiapas
- ***Telegonus chiriquensis*** Staudinger, 1875; Panama: Chiriquí
    - = *Aethilla weymeri* Plötz, 1882; ? [Panama: Chiriquí] | junior subjective synonym
- ***Telegonus colotrix*** Grishin, 2025; Colombia: Cauca
- ***Telegonus*** sp. undescribed #3, Steinhauser MS.; Bolivia
- ***Telegonus erana*** (Evans, 1952); Ecuador: Balzapamba
- ***Telegonus flavimargo*** Grishin, 2025; Costa Rica: Limón
- ***Telegonus*** sp. undescribed #4, Steinhauser MS.; Panama
- ***Telegonus flavifimbro*** Grishin, **sp. n.**; Colombia [likely eastern Andes]
- ***Telegonus tinda*** (Evans, 1952); Brazil: Pará
- ***Telegonus creteus*** (Cramer, 1780); Suriname
- ***Telegonus sobrasus*** Grishin, 2025; Brazil: Santa Catarina

parmenides species group

- ***Telegonus parmenides*** (Stoll, 1781); likely Suriname
- ***Telegonus panamus*** Grishin, 2025; Panama: Barro Colorado Island
- ***Telegonus tatus*** Grishin, 2025; Panama: Panamá
- ***Telegonus cretatus*** Hayward, 1939; Ecuador: Napo
    - = *Astraptes alfius alfius* Evans, 1952; Brazil: Amazonas | junior subjective synonym
- ***Telegonus adoba*** (Evans, 1952), **stat. nov.**; Brazil: Espírito Santo | was a subspecies of *T. cretatus*
- ***Telegonus chuchuvianus*** Grishin, 2025; Ecuador: Esmeraldas
- ***Telegonus meretrix*** (Hewitson, 1876); Ecuador
- ***Telegonus fulvimargo*** Grishin, 2025; Peru: Cuzco

latimargo species group

- ***Telegonus latimargo*** (Herrich-Schäffer, 1869)
    - *Telegonus latimargo aquila* (Evans, 1952); Colombia: Cauca
    - *Telegonus latimargo latimargo* (Herrich-Schäffer, 1869); Tropical America to USA
        - =‡ *Telegonus cartomes* Mabille & Boullet, 1912; no data | nomen nudum (proposed in synonymy)
        - = *Telegonus fabrici* Ehrmann, 1918; Venezuela: Caura Valley | junior subjective synonym
- ***Telegonus alardus*** (Stoll, 1790)
    - *Telegonus alardus latia* (Evans, 1952); Costa Rica
    - *Telegonus alardus alardus* (Stoll, 1790); Suriname
- ***Telegonus alardinus*** Grishin, 2025; Brazil: Rio de Janeiro
- ***Telegonus habana*** (Lucas, 1857); Cuba
- ***Telegonus heriul*** Mabille & Boullet, 1912; "Brazil" [Dominican Republic]
    - = *Telegonus antiquus* Skinner, 1920; Dominican Republic | junior subjective synonym
    - = *Telegonus domingensis* Joicey & Talbot, 1924; Dominican Republic | junior subjective synonym

galesus species group

- ***Telegonus subflavus*** Grishin, 2022; Ecuador: Chimborazo
    - =‡ *Telegonus galesus* form *subflavus* Williams, 1927; Ecuador: Chimborazo | infrasubspecific name
- ***Telegonus galesus*** Mabille, 1888; Peru: Chanchamayo
- ***Telegonus cassius*** (Evans, 1952); Costa Rica: Irazú

Urbanus (*Urbanoides*) *elmina* Evans, 1952 is confirmed from Colombia

In the original description of *Urbanus* (*Urbanoides*) *elma* Grishin, 2025 (type locality in Venezuela: Mérida) we provided a phylogenetic tree showing specimens of *Urbanus* (*Urbanoides*) *elmina* Evans, 1952 (type locality in Ecuador: Rio Pastaza) from Ecuador, Peru, and Argentina (Zhang et al. 2025a). The only specimen from Colombia (without further locality details) was the paratype of *U. elma*. To date, we have sequenced specimens of *U. elmina* from additional localities in Ecuador and also from western Colombia (Valle del Cauca and Cauca Departments), thus confirming this species from Colombia (Fig. 11). While we have not found more specimens of *U. elma*, we stumbled upon a specimen of a new species, which is described next.

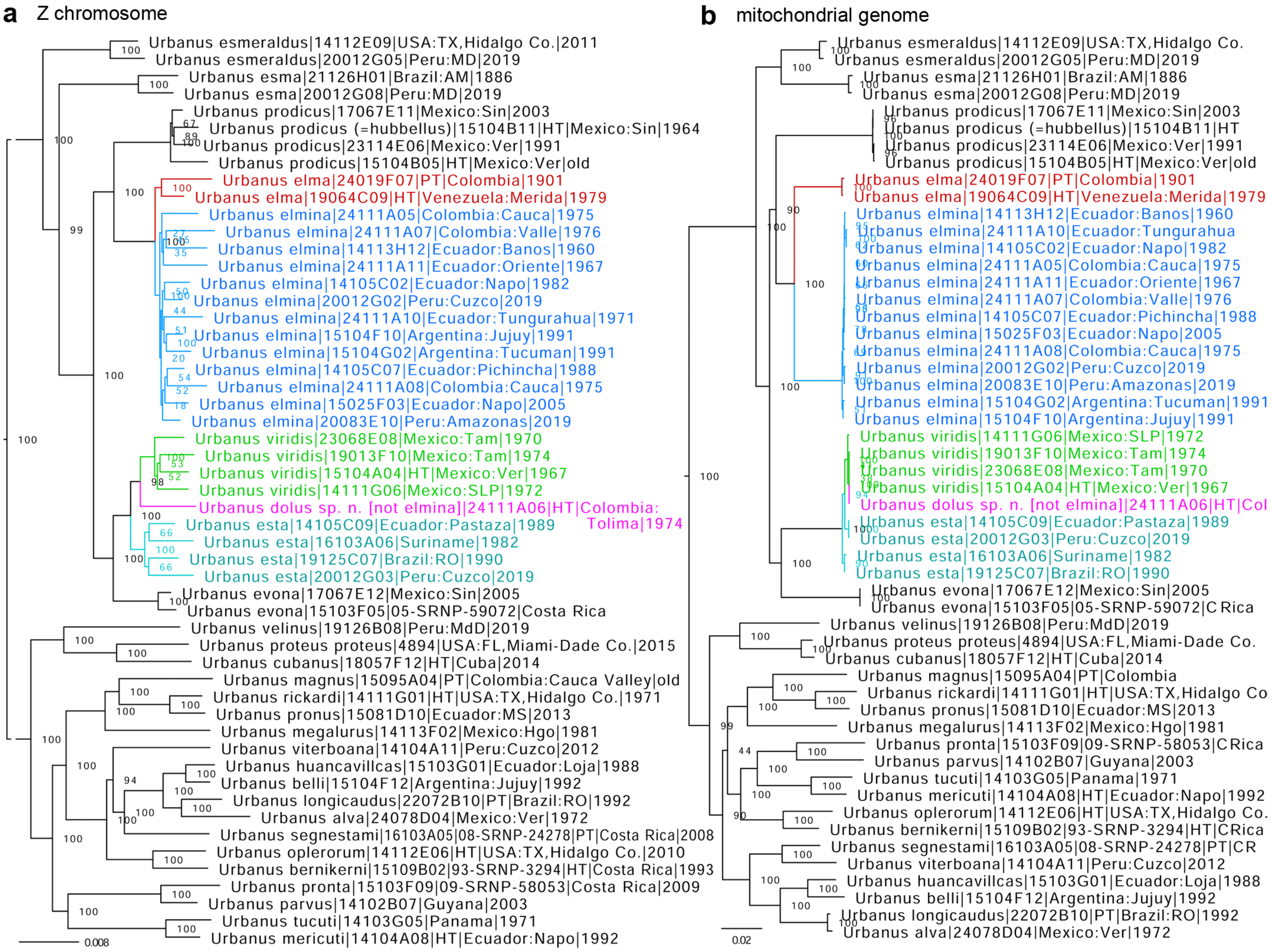


Fig. 11. Phylogenetic trees of selected *Urbanus* species constructed from protein-coding regions in: **a)** the Z chromosome, based on 548,172 positions, and **b)** the mitochondrial genome. Species discussed in the text are colored differently: *U. elma* (red), *U. elmina* (blue), *U. viridis* (green), *U. dolus* **sp. n.** (magenta), and *U. esta* (cyan). Gaps in branches indicate where vertical slices of the tree were removed to reduce its horizontal dimension (to allow an increase in the font size), i.e., branches with gaps are longer than shown.

Urbanus (*Urbanoides*) *dolus* Grishin, new species

http://zoobank.org/89F465B0-F9CE-4BB0-A902-257402570AD3

(Figs. 11 part, 12b, 13)

Definition and diagnosis. In an attempt to find additional specimens of the recently described *Urbanus* (*Urbanoides*) *elma* Grishin, 2025 (type locality in Venezuela: Mérida) (Fig. 12c), currently known only

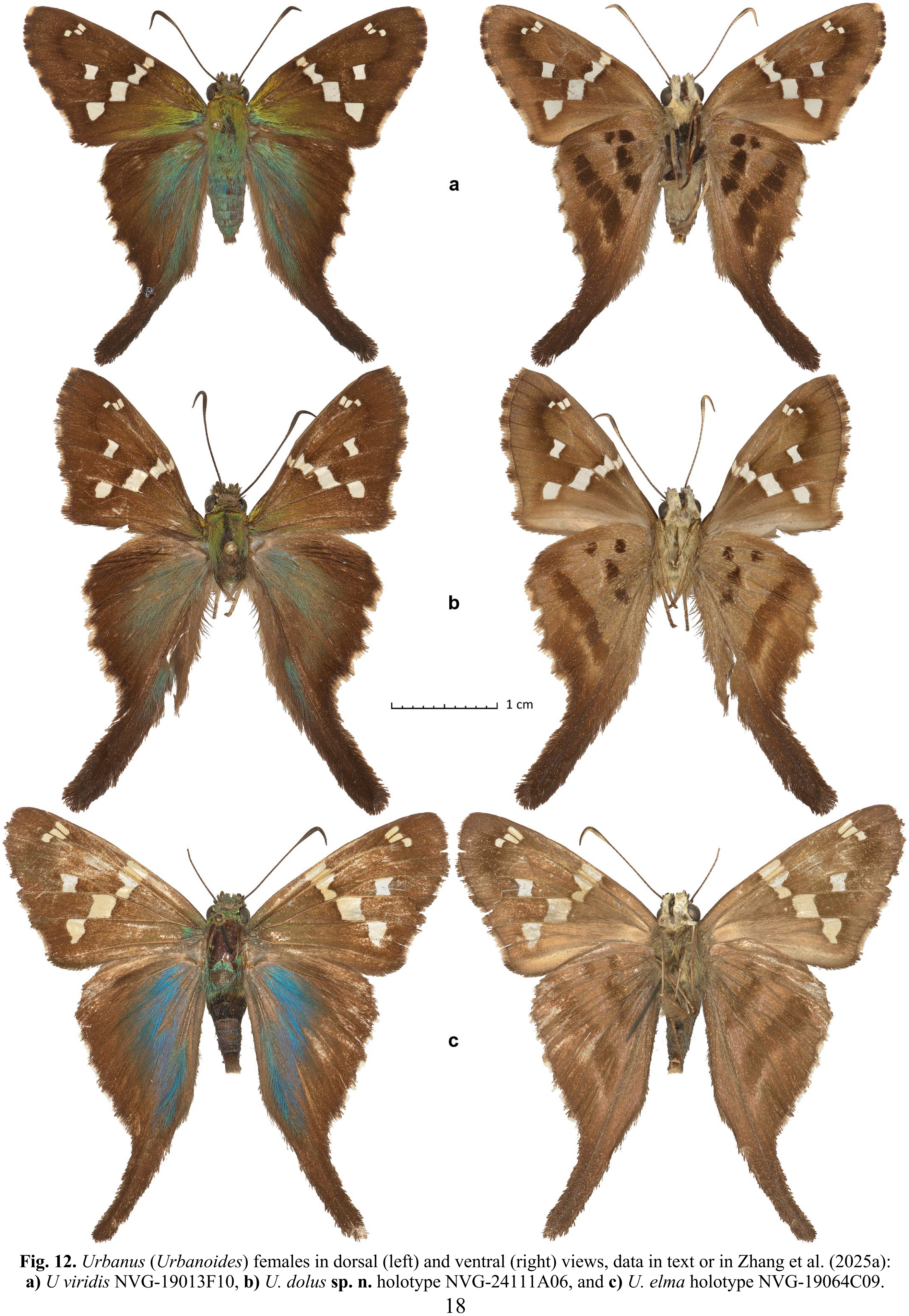


Fig. 12. *Urbanus* (*Urbanoides*) females in dorsal (left) and ventral (right) views, data in text or in Zhang et al. (2025a): **a)** *U viridis* NVG-19013F10, **b)** *U. dolus* **sp. n.** holotype NVG-24111A06, and **c)** *U. elma* holotype NVG-19064C09.

from the holotype (female) and the paratype (male), we have sequenced a female from Tolima, Colombia. This specimen, identified and illustrated (genitalia only) by Steinhauser (1981) as *Urbanus* (*Urbanoides*) *elmina* Evans, 1952 (type locality in Ecuador: Rio Pastaza), is instead sister to *Urbanus* (*Urbanoides*) *viridis* Freeman, 1970 (type locality in Mexico: Veracruz, holotype sequenced as NVG-15104A04) in the nuclear genome tree (*Urbanus* (*Urbanoides*) *esta* Evans, 1952 (type locality in Brazil: São Paulo) is sister to them both), but is genetically differentiated at the species level (Fig. 11). Therefore, this female represents a new species. Interestingly, COI barcodes do not differ among the three species: the new one, *U. viridis*, and *U. esta*, probably due to introgression. The new species keys to *U. elmina* in Evans (1952) (C.13.9) and Steinhauser (1981) and was included by them in that taxon, but differs from it and *U. elma* by the following combination of characters in females: the ventral hindwing with a narrower postdiscal brown band; four smaller, darker, and better separated from each other spots in the basal half; a stronger contrast between the marginal area of nearly ground color from the apex to the vein CuA_1 and the darker area from the vein CuA_1 through the tail; whiter (less yellow) semi-hyaline forewing spots; and a slightly rounder hindwing. The new species is more similar to its closer relatives *U. viridis* or *U. esta* in having a contrasting paler ventral hindwing margin from the apex to the vein CuA_1; better separated from each other four spots in the basal half of the ventral hindwing; whiter semi-hyaline forewing spots; and female genitalia with a moderately sclerotized lamella antevaginalis that has a well-developed central notch (Fig. 13); but differs from them by a more washed-out appearance of the ventral hindwing with paler bands and spots (but not as pale as in *U. elmina* and *U. elma*), the postdiscal band uniformly brown without paler veins crossing it, and smaller spots in the basal half. The lamella postvaginalis has a deeper, U-shaped central notch of more than a third of its length and its lobes have a convex distal margin on both sides. To facilitate comparison, we illustrate a female of its sister species, *U. viridis* (NVG-19013F10, Mexico: Tamaulipas, Sierra Cucharas, nr. rock quarry, eclosed on 3-Dec-1974, reared on *Desmodium neomexicanum* A. Gray, R. O. Kendall & C. A. Kendall leg. [TAMU]) (Fig. 12a). Due to the cryptic nature of this species, unrecognized males, and unexplored individual variation, most reliable identification is achieved by DNA, and a combination of the following base pairs is diagnostic in the nuclear genome: aly330.11.2:G174A, aly330.11.2:G510C, aly2828.8.1:T84C, aly2828.8.1:A291T, aly1038.10.3:C612T, aly2976.14.2:C150C (not T), aly6841.3.3:A116A (not G), aly6841.3.3:A499A (not G), aly6841.3.3:A867A (not T), aly1651.36.1:A1324A (not G). In the COI barcode, this species does not differ from either *U. viridis* or *U. esta*.

<u>Barcode sequence of the holotype.</u> Sample NVG-24111A06, GenBank PV892287, 658 base pairs:

```
AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAGTTGGTACTTCATTAAGATTAC
TTATTCGAACTGAATTAGGAACCCCTGGATCTTTAATTGGAGATGATCAAATTTATAAT
ACTATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAAT
TGGAGGATTTGGTAATTGATTAGTTCCCCTAATAATAGGAGCCCCTGACATAGCTTTCC
CCCGTATAAATAATATAAGATTTTGATTATTACCCCCTTCTTTAACTTTATTAATTTCA
AGAAGAATCGTTGAAAATGGTGCTGGTACTGGATGAACAGTTTATCCCCCTCTTTCATC
TAATATTGCCCATCAAGGAGCTTCTGTTGACTTAGCAATTTTTTCCCTACATCTTGCTG
GTATTTCATCTATTCTTGGAGCTATTAATTTTATTACTACAATTATTAATATACGAATT
AATAATTTATCTTTTGATCAAATACCTTTATTTGTATGAGCTGTAGGAATTACAGCATT
ATTATTATTATTATCTTTACCTGTTTTAGCGGGAGCTATCACTATATTATTAACTGATC
GAAATTTAAATACCTCCTTTTTTGACCCAGCAGGAGGAGGAGATCCTATTTTATATCAA
CATTTATTT
```

Type material. Holotype: ♀ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 12b (genitalia Fig. 13), bears the following five rectangular labels (2^{nd} handwritten, others printed with handwritten text shown in italics), four white: [COLOMBIA: TOLIMA | La Aurora, R. Cambrin | 1300 m.; *17.XI.*1974 | S. & L. Steinhauser], [Genit. Prep. | SRS-406], [A. C. Allyn | Acc. 1975-17], [DNA sample ID: | NVG-24111A06 | c/o Nick V. Grishin], and one red

Fig. 13. Female genitalia of *Urbanus* (*Urbanoides*) *dolus* **sp. n.** holotype NVG-24111A06 in ventral view, data in text: without bursa copulatrix (left, scale on the left) and complete (right, reduced to half the size, scale on the right).

[HOLOTYPE ♀ | *Urbanus* (*Urbanoides*) *dolus* Grishin]. Genitalia of the holotype, misidentified as *Urbanus elmina*, were illustrated in fig. 58 by Steinhauser (1981).

Type locality. Colombia: Tolima, La Aurora, Río Cambrín, elevation 1300 m.

Etymology. In Greek, δόλος (dólos) means deceit, treachery, guile, or craftiness; the same meaning carries to Latin *dolus*. The name is given for the deceitful appearance of this species, which is sister to *U. viridis*, but in the ventral hindwing pattern looks more similar to *U. elmina* or *U. elma* and was identified as *U. elmina* by Steinhauser (1981). The name is an adjective.

Distribution. Currently known only from the holotype collected on the western slopes of the eastern Andes of Colombia.

Subspecies of *Burnsius communis* (Grote, 1872)

Currently, *Burnsius communis* (Grote, 1872) (type locality in USA: central Alabama) is partitioned into two subspecies: the nominate, distributed throughout most of the range from Canada to Mexico; and *Burnsius communis albescens* (Plötz, 1884) (type locality in Mexico), known from the southern parts of the range (e.g., Oaxaca and Veracruz in Mexico). In line with this treatment, *B. communis albescens* is a confidently supported sister to all other populations (98% bootstrap value) in the nuclear genome tree constructed from more than 10 million positions in autosome protein-coding genes (Fig. 14a). However, due to their overall lack of prominent genetic differentiation and continuing gene flow within a species, subspecies do not always stand out as major clades in phylogenetic trees. Accordingly, the clade of *B. communis albescens* specimens, although supported with 100% ultra-fast bootstrap value (Minh et al. 2013), is found within the nominate clade in the Z chromosome tree (Fig. 14b), likely due to poor

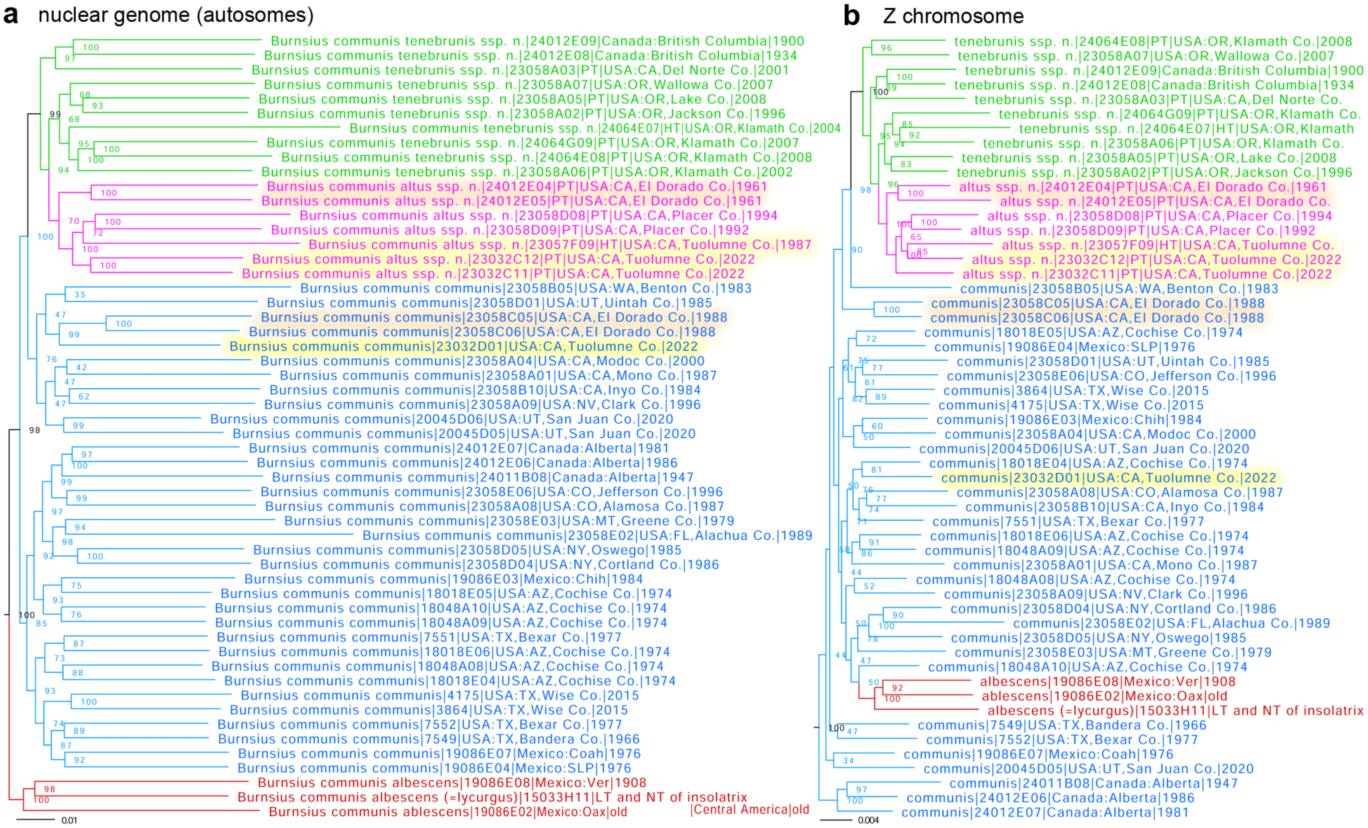


Fig. 14. Phylogenetic trees of *Burnsius communis* specimens constructed from protein-coding regions in: **a)** the nuclear genome (autosomes), based on 10,128,060 positions, and **b)** the Z chromosome, based on 303,834 positions. The mitochondrial genome tree is not shown because it does not separate several species of *Burnsius*. Different subspecies are colored differently: *B. communis tenebrunis* **ssp. n.** (green), *B. communis altus* **ssp. n.** (magenta), *B. communis communis* (blue), and *B. communis albescens* (red). Labels of specimens from different clades collected in El Dorado and Tuolumne Counties, California, are highlighted in orange and yellow, respectively.

phylogenetic resolution (low support for most bifurcations) caused by DNA similarity and gene flow. We note that mitochondrial DNA does not consistently segregate several species of *Burnsius* Grishin, 2019 (type species *Syricthus* [sic] *communis* Grote, 1872), such as *B. communis*, *B. albezens* Grishin, 2022 (type locality in USA: AZ, Cochise Co.), and *B. burnsi* Grishin, 2022 (type locality in Mexico: Veracruz), and its utility is equally limited for subspecies delimitation.

Additional sequencing of *B. communis* specimens across the range revealed that specimens from Stanislaus National Forest in Tuolumne County, California, were partitioned between different clades (Fig. 14 yellow highlight). While several specimens were in a confidently supported (99%–100% bootstrap value) nuclear genome clade of specimens from the northwestern part of the range, one specimen was in a clade that included more western and southern specimens. This latter specimen was collected at the same locality and on the same day with one of the specimens from the northwestern clade (near Mill Creek Campground, elevation 6525', GPS 38.3126, −119.9398, 21-Jul-2022, W. R. Dempwolf leg.). These specimens were different in size and wing pattern (Fig. 15h vs. Fig. 15l). Moreover, a similar scenario was later found for four specimens of the same size from south of Lake Tahoe, El Dorado County, California, with two being in the northwestern clade and two (collected several miles to the east) falling in the southeastern clade (Fig. 14 orange highlight; Fig. 15j, k, m, n).

Therefore, the northwestern and southeastern clades represent different taxa. Although it is possible that they are species-level taxa, it is also possible that they may be subspecies that intergrade at certain localities and elevations. Currently, several taxa with similar relationships are treated as subspecies (type localities in parentheses): *Hesperia colorado sublima* A. Warren & Calhoun, 2015 (USA: Colorado, Clear Creek Co.) vs. *Hesperia colorado colorado* (Scudder, 1874) (USA: Colorado, Lake Co.) and *Apodemia virgulti dialeucoides* J. Emmel, T. Emmel & Pratt, 1998 vs. *Apodemia virgulti nigrescens* J. Emmel & T. Emmel, 1998 (both USA: California, San Bernardino Co.) (Pelham 2023). Hence, and due to the lack of prominent genetic differentiation characteristic of species, the new taxa of *Burnsius* are conservatively described here as subspecies of *B. communis*, pending more detailed analyses and further studies. Warren (2005) reviewed Oregon populations and also noted phenotypic differences among them.

Burnsius communis tenebrunis **Grishin, new subspecies**

http://zoobank.org/E738CD38-E871-4B6A-A77E-4C6C5ED5969E

(Figs. 14 part, 15a–d, 16a–b)

Definition and diagnosis. Genomic analysis reveals that populations from the Pacific Northwest, currently identified as *Burnsius communis communis* (Grote, 1872) (type locality in USA: central Alabama), belong to a distinct and strongly supported (99%–100% ultra-fast bootstrap values) clade in the nuclear genome trees, genetically differentiated from others at the subspecies level (Fig. 14 green). Therefore, they represent a new subspecies that keys to "*Pyrgus communis communis*" (G.1.10(a)) in Evans (1953), but differs from it and other relatives by the following combination of characters: a costal fold in males; the harpe dorsally with two prongs (Fig. 16a), but the valva is typically narrower and with a less pronounced costal hump than in specimens from the rest of the range—see Burns (2000) for illustrations—and in these characters is intermediate towards *Burnsius albezens* Grishin, 2022 (type locality in USA: AZ, Cochise Co.); the wings are usually darker above (especially in females, Fig. 15b) with smaller white spots and areas, and with better-defined dark framing of ventral spots, bands, and veins; and a less olive, duller tone of the ventral bands. Due to significant and uncharacterized in many parts of the range individual variation, this subspecies is best identified by DNA, with diagnostic base pairs in the nuclear genome: aly18826.4.1:C138T, aly383.20.2:C864T, aly383.20.2:G1528T, aly383.20.2:C993T, aly1313.19.2:C189T, aly451.12.5:T86T (not G), aly276378.20.2:A24A (not G), aly276378.20.2:C57C (not T), aly276378.20.2:T60T (not C), aly1656.6.2:C1106C (not G); but no COI barcode differences.

Barcode sequence of the holotype. Sample NVG-24064E07, GenBank PV892288, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACTTCTTTAAGTTTATTAATTCGAACTGAATTAGGAAATCCCGGCTCATTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCACATGCTTTCATTATAATTTTTTTTATAGTCATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATACTAGGAGCTCCAGATATAGCATTCCCCCGTA
TAAATAACATAAGATTTTGATTATTACCCCCTTCATTAACATTACTTATTTCAAGAAGTATTGTAGAAAACGGTGCAGGAACTGGATGAACAGTTTACCCCCCATTATCAGCTAATATTGC
TCATCAAGGTTCTTCTGTTGATTTAGCTATTTTTTCATTACATTTAGCAGGAATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACAATTATTAATATACGTATTAGAAATTTATCA

Fig. 15. Selected specimens of *Burnsius communis* in dorsal (left) and ventral (right) views, data in text or below. **a–d)** *Burnsius communis tenebrunis* **ssp. n.**: **a–b)** types from USA, Oregon, Klamath Co.: **a)** holotype ♂ NVG-24064E07 and **b)** paratype ♀ NVG-23058A06 and **c–d)** non-type ♂♂ from Canada, British Columbia: **c)** NVG-24012E08 (slightly aberrant) and **d)** NVG-24012E09. **e–f)** *Burnsius communis communis* ♂♂ from Canada, Alberta, E. M. Pike leg. [CNC]: **e)** NVG-24012E07 Green Is., 18-Aug-1981 and **f)** NVG-24012E06 Finnegan, 18-Aug-1986. **g–k)** *Burnsius communis altus* **ssp. n.** from USA, California: **g–i)** Tuolumne Co.: **g)** holotype ♂ NVG-23057F09 and **h–i)** paratypes: **h)** ♀ NVG-23032C12 and **i)** ♀ NVG-23032C11 and **j–k)** El Dorado Co. paratypes: **j)** ♂ NVG-24012E04 and **k)** ♂ NVG-24012E05. **l–m)** *Burnsius communis communis* specimens sympatric or collected near *B. communis altus* **ssp. n.** from USA, California: **l)** ♀ NVG-23032D01, WRD 22433 Tuolumne Co. (data exactly as NVG-23032C12, see text) and **m–n)** ♂♂ from El Dorado Co., S of Lake Tahoe, Cold Creek, D. L. Bauer leg., 8-Sep-1988 [MGCL]: **m)** NVG-23058C05 and **n)** NVG-23058C06.

Fig. 16. Male genitalia of *Burnsius communis* subspecies, holotypes in: **a, c)** left lateral and **b, d)** dorsal views, data in text: **a–b**) *B. communis tenebrunis* **ssp. n.** NVG-24064E07 and **c–d**) *B. communis altus* **ssp. n.** NVG- 23057F09.

TTTGATCAAATACCTTTATTTGTTTGAGCAGTAGGTATTACAGCTTTATTATTATTATTATCATTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTAAATACATCATTTTTTGATCCTGCTGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 15a (genitalia Fig. 16a, b), bears the following four printed rectangular labels (handwritten text shown in italics), three white: [*W. of Little Deschutes Riv.* | *near Crescent El. 4400'* | *Klamath Co, Oregon* | *August 21, 2004* | COLLECTORS JUNE | & FLOYD PRESTON], [MGCL Accession | 2010-33 | J.&F. Preston], [DNA sample ID: | NVG-24064E07 | c/o Nick V. Grishin], [genitalia: | NVG241111-10 | c/o Nick V. Grishin] and one red [HOLOTYPE ♂ | Burnsius communis | tenebrunis Grishin].

Paratypes: 3♂♂ and 3♀♀: USA, J. & F. Preston leg. [MGCL]: Oregon: 1♂ NVG-23058A02 Jackson Co., 3.2 mi S of OR-66 on Soda Mt. Rd., 5000’, 31-May-1996; Klamath Co., Deschutes National Forest, Little Deschutes River nr. Mowich, 4700’: 1♀ NVG-23058A06 29-Jun-2002 (Fig. 15b), 1♀ NVG-24064G09 27-Jun-2007, and 1♂ NVG-24064E08 29-Jun-2008; and 1♂ NVG-23058A05 Lake Co., Warner Mts., Fremont National Forest, FR3915 4.4 rd. mi S of Camas Creek, 6000’, 7-Jul-2008; and California: 1♀ NVG-23058A03 Del Norte Co., 2 rd. mi E of Rowdy Creek Rd. on Low Divide Rd., 1600’, 1-Sep-2001.

Other specimens: Due to genetic similarity (Fig. 14), we currently attribute the following three sequenced specimens to this subspecies but exclude them from the type series, as they exhibit stronger phenotypic differences—being paler and larger—compared to the population at the type locality: British Columbia [CNC]: 1♂ NVG-24012E09, CNCLEP_00163304 Kaslo, 19-Jun-1900, J. W. Cockle (Fig. 15d) and 1♂ NVG-24012E08, CNCLEP_00163301 Fernie, 12-Jun-1934, H. B. Leech (Fig. 15c) and 1♀ NVG-23058A07 USA, Oregon, Wallowa Co., 12 road mi NE of Joseph, on road to Imnaha, 3700’, 26-Jul-2007, J. & F. Preston leg. [MGCL].

Type locality. USA: Oregon, Klamath Co., Deschutes National Forest, west of Little Deschutes River, nr. Crescent, 4400’.

Etymology. In Latin, *tenebrosus* means dark, gloomy, or shadowy, and *brunneus* means brown. The name is formed as a fusion: *tene*[brosus] + *brun*[neus] + [commun]*is*, given for the darker and browner (not greener) aspect of this subspecies, and is treated as an adjective.

Distribution. From British Columbia (Canada), through Oregon to northwestern California (USA).

Burnsius communis altus Grishin, new subspecies

http://zoobank.org/5DF6A207-4270-414E-91DB-8104C19B2452

(Figs. 14 part, 15g–k, 16c–d)

Definition and diagnosis. Genomic analysis reveals that populations from the central Sierra Nevada, California, currently assigned to *Burnsius communis communis* (Grote, 1872) (type locality in USA: central Alabama), belong to a distinct and strongly supported (99%-100% ultra-fast bootstrap values) clade in the nuclear genome trees (together with the northwestern subspecies described above) genetically differentiated from others at the subspecies level (Fig. 14 magenta vs. green) and phenotypically different from them. Therefore, they represent a new subspecies that keys to "*Pyrgus communis communis*" (G.1.10(a)) in Evans (1953), but differs from it and other relatives by the following combination of characters: a costal fold in males; the harpe dorsally with two prongs (Fig. 16c) and the valva usually broader than in the northwestern new subspecies described above, with a pronounced costal hump typical of specimens from most of the range—see Burns (2000) for illustrations; the wings usually darker above with smaller white spots and areas (Fig. 15g–k), but paler than in the northwestern new subspecies (Fig. 15a–d), and with better-defined dark framing of ventral spots, bands, and veins; and a less olive, duller tone of the ventral bands. Due to significant and poorly characterized individual variation, this subspecies is best identified by DNA, with diagnostic base pairs in the nuclear genome: aly451.12.5:T86G, aly276378.20.2:A24G, aly276378.20.2:C57T, aly276378.20.2:T60C, aly1656.6.2:C1106G; but no barcode differences.

Barcode sequence of the holotype. Sample NVG-23057F09, GenBank PV892289, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACTTCTTTAAGTTTATTAATTCGAACTGAATTAGGAAATCCCGGCTCATTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCACATGCTTTCATTATAATTTTTTTTATAGTCATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATACTAGGAGCTCCAGATATAGCATTCCCCCGTA
TAAATAACATAAGATTTTGATTATTACCCCCTTCATTAACATTACTTATTTCAAGAAGTATTGTAGAAAACGGTGCAGGAACTGGATGAACAGTTTACCCCCCATTATCAGCTAATATTGC
TCATCAAGGTTCTTCTGTTGATTTAGCTATTTTTTCATTACATTTAGCAGGAATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACAATTATTAATATACGTATTAGAAATTTATCA
TTTGATCAAATACCTTTATTTGTTTGAGCAGTAGGTATTACAGCTTTATTATTATTATTATCATTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTAAATACAT
CATTTTTTGATCCTGCTGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 15g, (genitalia Fig. 16c, d), bears the following six printed rectangular labels, five white: [CALIF.: Tuolumne Co. | 4 mi. S of Mill Creek | at CA Hwy. 108, 6500 | ft.; 30.vi.1987 | L.D. & J.Y. Miller | sta. no. 4], [Allyn Museum | Acc. 1987–8], [DNA sample ID: | NVG-23057F09 | c/o Nick V. Grishin], [DNA sample ID: | NVG-24067E03 | c/o Nick V. Grishin], [genitalia: | NVG241111-29 | c/o Nick V. Grishin] and one red [HOLOTYPE ♂ | Burnsius communis | altus Grishin]. The first DNA sample refers to the extraction from a leg (sequenced) and the second is from the abdomen (stored) prior to genitalia dissection. **Paratypes:** 3♂♂ and 3♀♀: USA, California: Placer Co., B. O'Hara leg. [MGCL]: 1♂ NVG-23058D09 Soda Springs Rd. 2.9-4.1 mi S. of Donner Pass Rd., 27-Jul-1992 and 1♂ NVG-23058D08 Squaw Valley Ski Area, 8200-8500', 6-Jun-1994; El Dorado Co. [CNC]: 1♂ NVG-24012E04, CNCLEP_00163011 Fallen Leaf, 13-Jul-1961, J. G. Chillcott leg. (Fig. 15j) and 1♀ NVG-24012E05, CNCLEP_00163017 Echo Lake, 13-Jul-1961, B. H. Poole leg (Fig. 15k); and Tuolumne Co., Stanislaus National Forest, 21-Jul-2022, W. R. Dempwolf leg. [WRD]: 1♀ NVG-23032C11, WRD 22431 near Niagara Campground, 6870', 38.3206, −119.9114 (Fig. 15i) and 1♀ NVG-23032C12, WRD 22432 near Mill Creek Campground, 6525', 38.3126, −119.9398 (Fig. 15h).

Type locality. USA: California, Tuolumne Co., 4 mi south of Mill Creek at SR 108, elevation 6500 ft.

Etymology. In Latin, *altus* means high, deep, or tall, and is given for the typical habitat of this subspecies at higher elevations. The name is an adjective.

Distribution. Currently known from the central Sierra Nevada in California, USA (Placer, El Dorado, and Tuolumne Counties), where it comes close to and may overlap at times with the nominate subspecies, especially at lower elevations.

Additional specimens of *Heliopetes* (*Heliopetes*) *acuta* Grishin, 2024 confirm it as a species-level taxon

Genomic analysis of *Heliopetes* Billberg, 1820 (type species *Papilio niveus* Cramer, 1775, which is a junior subjective synonym of *Papilio arsalte* Linnaeus, 1758) reveals four additional specimens of *Heliopetes* (*Heliopetes*) *acuta* Grishin, 2024 (type locality Mexico, Oaxaca, Candelaria Loxicha), all from the type locality and collected by E. C. Welling (Fig. 17 red), thus confirming that it is a species-level taxon and not an unusual single specimen. In all three trees, *H. acuta* is sister to the clade consisting of three species: *Heliopetes* (*Heliopetes*) *lana* Grishin, 2023 (type locality in Guatemala), *Heliopetes* (*Heliopetes*) *alana* (Reakirt, 1868) (type locality in Colombia), and *Heliopetes* (*Heliopetes*) *chimbo* Evans, 1953 (type locality in Ecuador: Chimbo), and, therefore, is the most distinct species in this group. Here, we illustrate male genitalia of *H. acuta* (Fig. 18) and they differ from those of *H. lana*, a species closest in distribution or possibly even sympatric with it (see figs. 302–303 in Zhang et al. (2023a) for its genitalia photographs), by a terminally rounder and flatter (shell-shaped in the dorsal half) harpe, which is not prominently turning inward and bears coarser serrations mostly along its dorsoposterior margin; a more robust ampulla; and slightly narrower, more separated uncus arms.

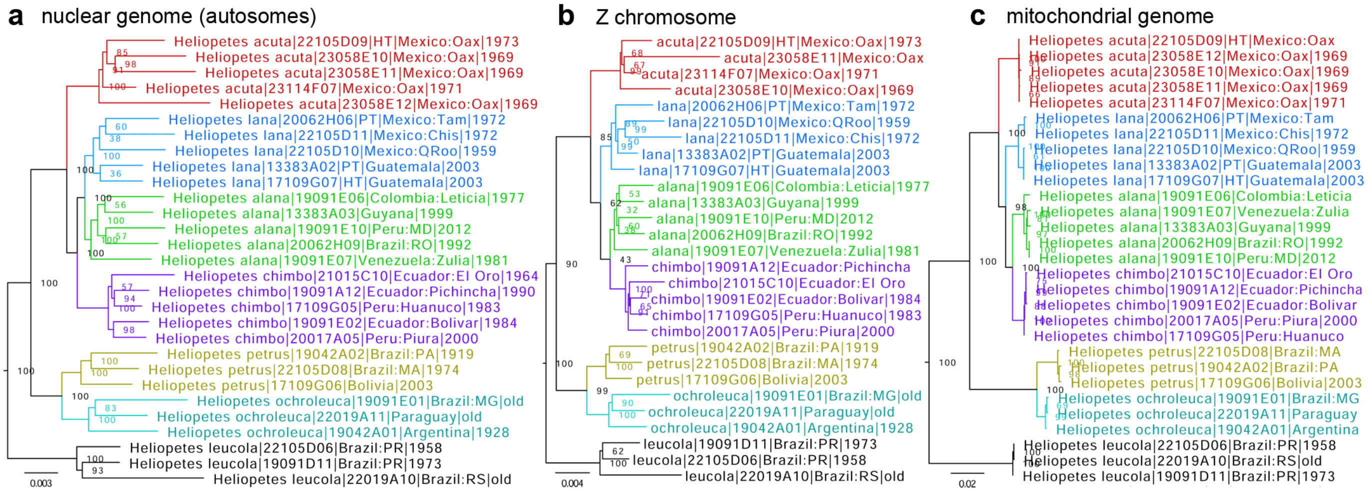


Fig. 17. Phylogenetic trees of selected *Heliopetes* (*Heliopetes*) *alana* relatives constructed from protein-coding regions in: **a)** the nuclear genome (autosomes), based on 887,994 positions, **b)** the Z chromosome, based on 261,456 positions, and **c)** the mitochondrial genome. Different species are colored differently: *H. acuta* (red), *H. lana* (blue), *H. alana* (green), *H. chimbo* (purple), *H. petrus* (Hübner, [1819]) (olive), *H. ochroleuca* J. Zikán, 1938 (cyan), and *H. leucola* (Hewitson, 1868) (black).

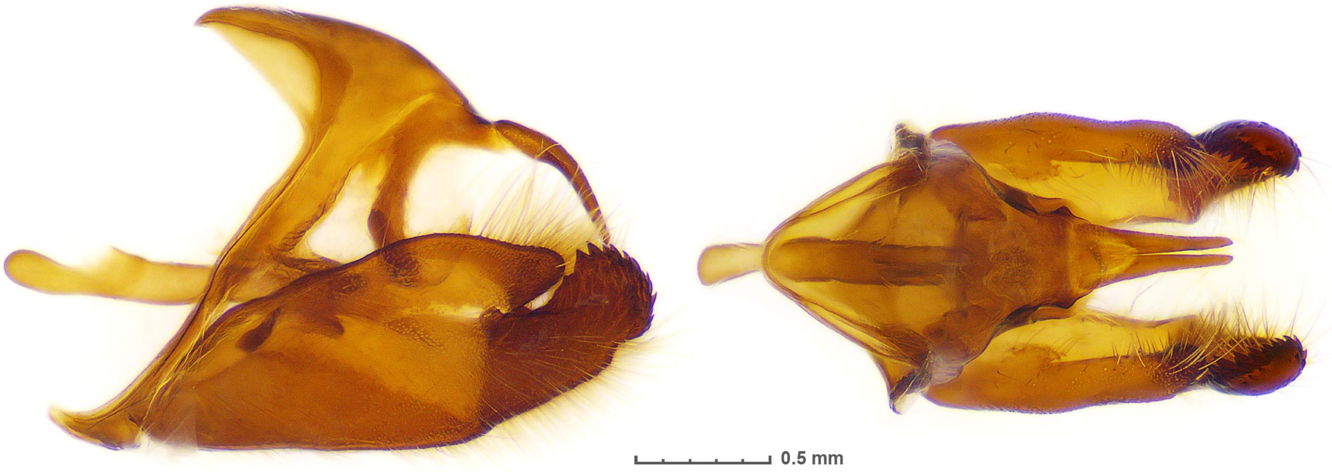


Fig. 18. Male genitalia of *Heliopetes* (*Heliopetes*) *acuta* specimen NVG-23114F07 from Mexico: Oaxaca, Candelaria Loxicha, 31-Aug-1971, E. C. Welling leg., genitalia NVG241118-79 [CMNH] in left lateral (left) and dorsal (right) views.

Ochlodes (*Ochluma*) *sylvanoides* group

Previously treated as a single species *Ochlodes sylvanoides* (Boisduval, 1852) (type locality in USA: California, Plumas Co.), this species group is currently placed in the subgenus *Ochluma* Grishin, 2025 (type species *Hesperia yuma* W. H. Edwards, 1873) and, as here defined, consists of three genetically differentiated species: *Ochlodes* (*Ochluma*) *santacruza* J. Scott, 1981 (type locality USA: California, Santa Barbara Co., Santa Cruz Island), *O. sylvanoides*, and *Ochlodes* (*Ochluma*) *napa* (W. H. Edwards, 1865) (type locality in USA: Colorado, Clear Creek Co.) (Zhang et al. 2023b). We expanded genomic sequencing of this group by sampling additional specimens across the range from southwestern Canada through all 13 western states of the USA to Baja California, Mexico (Fig. 19). The results confirm the phylogenetic separation of the group into three species and reveal additional insights, some of which are unexpected.

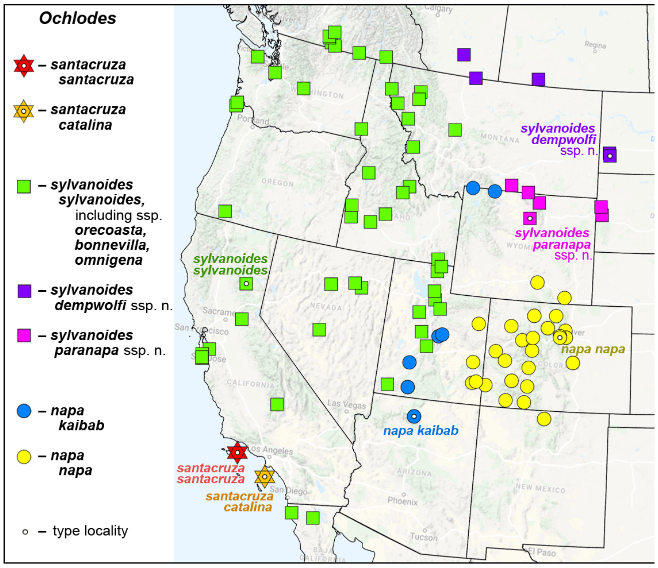
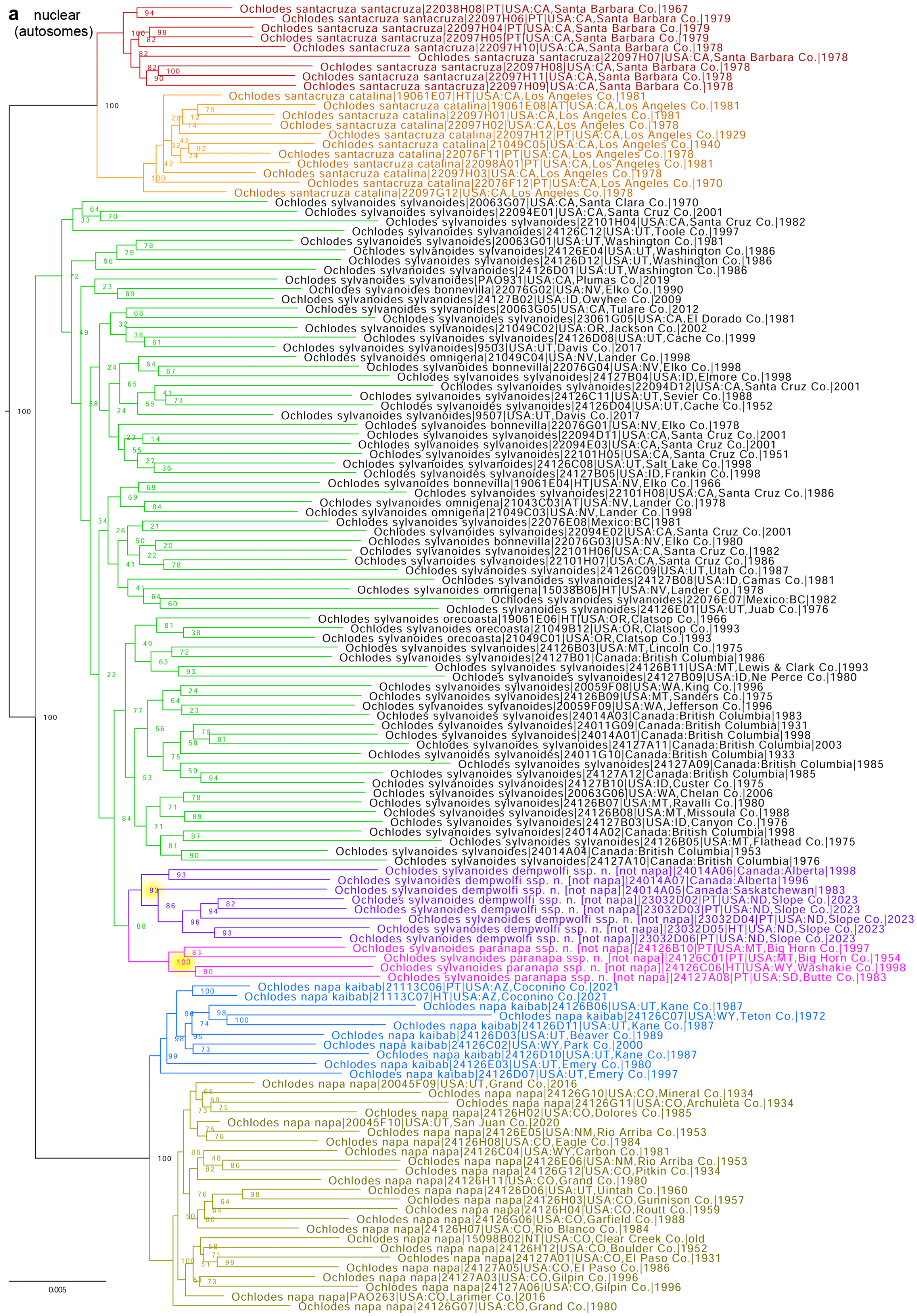


Fig. 19. A map of sequenced specimens from the *Ochlodes sylvanoides* group. Species are shown as symbols of different shapes and subspecies are denoted by color: *O. santacruza* (stars), *O. sylvanoides* (squares), and *O. napa* (circles); *O. santacruza santacruza* (red), *O. santacruza catalina* (orange), *O. sylvanoides sylvanoides* including *orecoasta*, *bonnevilla*, and *omnigena* (green), *O. sylvanoides dempwolfi* **ssp. n.** (violet), *O. sylvanoides paranapa* **ssp. n.** (magenta), *O. napa kaibab* (blue), and *O. napa napa* (yellow). The type localities are marked with tiny white circles inside symbols, and names of corresponding subspecies are placed nearby.

First, the nominate subspecies of *O. napa* (Fig. 19 yellow circles) is restricted to the mountainous region of Colorado and its immediate neighborhood in southeastern Wyoming, eastern Utah, and northern New Mexico. Second, *Ochlodes* (*Ochluma*) *napa kaibab* Grishin, 2023 (type locality in USA: Arizona, Coconino Co.) (Fig. 19, blue circles) has a much wider distribution than anticipated and spreads as a narrow strip north from the type locality through central Utah, reaching northwestern Wyoming. This subspecies of *O. napa*, genetically differentiated from the nominate, represents populations between it and neighboring *O. sylvanoides* while being phylogenetically associated with the former. It is also possible that the populations of *O. napa* in Wyoming, Utah, and Arizona are best partitioned into several subspecies. They have different, albeit more closely related, mitogenome haplotypes (Fig. 20b) and show different levels of introgression with other taxa. For instance, specimens of *O. napa kaibab* from Emery County, Utah, introgress stronger with *O. napa napa* and thus are placed near the base of the nuclear genome tree (Fig. 20a) and possess mitochondrial genomes of *O. napa napa*. Nevertheless, specimens shown as blue circles in Fig. 19 are in the clade with *O. napa kaibab*, and we presently treat them as this subspecies. Furthermore, the populations of *O. napa kaibab* in Utah are coming close to and may even be sympatric with *O. sylvanoides*, a question to be addressed in future studies.

Third, paler-colored populations to the west of the main Rocky Mountains chain north of Central Wyoming (Fig. 19 magenta and violet squares) traditionally associated with *O. napa* do not belong to this species and are *O. sylvanoides* instead. Due to their wing pattern differences that resulted in this misidentification, these populations are described below as two new subspecies that are somewhat differentiated genetically from other *O. sylvanoides* populations. These populations of *O. sylvanoides* are geographically close to *O. napa kaibab* in northwestern Wyoming and south-central Montana, another region to study interactions between the two species *O. napa* and *O. sylvanoides*.

Fourth, phylogenetic analysis did not reveal prominent genetic differences of previously described *O. sylvanoides* subspecies: *Ochlodes sylvanoides orecoasta* J. Scott, 1981 (type locality in USA: Oregon, Clatsop Co.), *Ochlodes sylvanoides bonnevilla* J. Scott, 1981 (type locality in USA: Nevada, Elko Co.),


a nuclear (autosomes)
Ochlodes santacruza santacruza|22038H08|PT|USA:CA,Santa Barbara Co.|1967
Ochlodes santacruza santacruza|22097H06|PT|USA:CA,Santa Barbara Co.|1979
Ochlodes santacruza santacruza|22097H04|PT|USA:CA,Santa Barbara Co.|1979
Ochlodes santacruza santacruza|22097H05|PT|USA:CA,Santa Barbara Co.|1979
Ochlodes santacruza santacruza|22097H10|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H07|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H08|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H11|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H09|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza catalina|19061E07|HT|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|19061E08|AT|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|22097H01|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|22097H02|USA:CA,Los Angeles Co.|1978
Ochlodes santacruza catalina|22097H12|PT|USA:CA,Los Angeles Co.|1929
Ochlodes santacruza catalina|21049C05|PT|USA:CA,Los Angeles Co.|1940
Ochlodes santacruza catalina|22076F11|PT|USA:CA,Los Angeles Co.|1978
Ochlodes santacruza catalina|22098A01|PT|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|22097H03|USA:CA,Los Angeles Co.|1978
Ochlodes santacruza catalina|22076F12|PT|USA:CA,Los Angeles Co.|1970
Ochlodes santacruza catalina|22097G12|USA:CA,Los Angeles Co.|1978
Ochlodes sylvanoides sylvanoides|20063G07|USA:CA,Santa Clara Co.|1970
Ochlodes sylvanoides sylvanoides|22094E01|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|22101H04|USA:CA,Santa Cruz Co.|1982
Ochlodes sylvanoides sylvanoides|24126C12|USA:UT,Toole Co.|1997
Ochlodes sylvanoides sylvanoides|20063G01|USA:UT,Washington Co.|1981
Ochlodes sylvanoides sylvanoides|24126E04|USA:UT,Washington Co.|1986
Ochlodes sylvanoides sylvanoides|24126D12|USA:UT,Washington Co.|1986
Ochlodes sylvanoides sylvanoides|24126D01|USA:UT,Washington Co.|1986
Ochlodes sylvanoides sylvanoides|PAO931|USA:CA,Plumas Co.|2019
Ochlodes sylvanoides bonnevilla|22076G02|USA:NV,Elko Co.|1990
Ochlodes sylvanoides sylvanoides|24127B02|USA:ID,Owyhee Co.|2009
Ochlodes sylvanoides sylvanoides|20063G05|USA:CA,Tulare Co.|2012
Ochlodes sylvanoides sylvanoides|23061G05|USA:CA,El Dorado Co.|1981
Ochlodes sylvanoides sylvanoides|21049C02|USA:OR,Jackson Co.|2002
Ochlodes sylvanoides sylvanoides|24126D08|USA:UT,Cache Co.|1999
Ochlodes sylvanoides sylvanoides|9503|USA:UT,Davis Co.|2017
Ochlodes sylvanoides omnigena|21049C04|USA:NV,Lander Co.|1998
Ochlodes sylvanoides bonnevilla|22076G04|USA:NV,Elko Co.|1998
Ochlodes sylvanoides sylvanoides|24127B04|USA:ID,Elmore Co.|1998
Ochlodes sylvanoides sylvanoides|22094D12|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|24126C11|USA:UT,Sevier Co.|1988
Ochlodes sylvanoides sylvanoides|24126D04|USA:UT,Cache Co.|1952
Ochlodes sylvanoides sylvanoides|9507|USA:UT,Davis Co.|2017
Ochlodes sylvanoides bonnevilla|22076G01|USA:NV,Elko Co.|1978
Ochlodes sylvanoides sylvanoides|22094D11|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|22094E03|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|22101H05|USA:CA,Santa Cruz Co.|1951
Ochlodes sylvanoides sylvanoides|24126C08|USA:UT,Salt Lake Co.|1998
Ochlodes sylvanoides sylvanoides|24127B05|USA:ID,Franklin Co.|1998
Ochlodes sylvanoides bonnevilla|19061E04|HT|USA:NV,Elko Co.|1966
Ochlodes sylvanoides sylvanoides|22101H08|USA:CA,Santa Cruz Co.|1986
Ochlodes sylvanoides omnigena|21043C03|AT|USA:NV,Lander Co.|1978
Ochlodes sylvanoides omnigena|21049C03|USA:NV,Lander Co.|1998
Ochlodes sylvanoides sylvanoides|22076E08|Mexico:BC|1981
Ochlodes sylvanoides sylvanoides|22094E02|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides bonnevilla|22076G03|USA:NV,Elko Co.|1980
Ochlodes sylvanoides sylvanoides|22101H06|USA:CA,Santa Cruz Co.|1982
Ochlodes sylvanoides sylvanoides|22101H07|USA:CA,Santa Cruz Co.|1986
Ochlodes sylvanoides sylvanoides|24126C09|USA:UT,Utah Co.|1987
Ochlodes sylvanoides sylvanoides|24127B08|USA:ID,Camas Co.|1981
Ochlodes sylvanoides omnigena|15038B06|HT|USA:NV,Lander Co.|1978
Ochlodes sylvanoides sylvanoides|22076E07|Mexico:BC|1982
Ochlodes sylvanoides sylvanoides|24126E01|USA:UT,Juab Co.|1976
Ochlodes sylvanoides orecoasta|19061E06|HT|USA:OR,Clatsop Co.|1966
Ochlodes sylvanoides orecoasta|21049B12|USA:OR,Clatsop Co.|1993
Ochlodes sylvanoides orecoasta|21049C01|USA:OR,Clatsop Co.|1993
Ochlodes sylvanoides sylvanoides|24126B03|USA:MT,Lincoln Co.|1975
Ochlodes sylvanoides sylvanoides|24127B01|Canada:British Columbia|1986
Ochlodes sylvanoides sylvanoides|24126B11|USA:MT,Lewis & Clark Co.|1993
Ochlodes sylvanoides sylvanoides|24127B09|USA:ID,Ne Perce Co.|1980
Ochlodes sylvanoides sylvanoides|20059F08|USA:WA,King Co.|1996
Ochlodes sylvanoides sylvanoides|24126B09|USA:MT,Sanders Co.|1975
Ochlodes sylvanoides sylvanoides|20059F09|USA:WA,Jefferson Co.|1996
Ochlodes sylvanoides sylvanoides|24014A03|Canada:British Columbia|1983
Ochlodes sylvanoides sylvanoides|24011G09|Canada:British Columbia|1931
Ochlodes sylvanoides sylvanoides|24014A01|Canada:British Columbia|1998
Ochlodes sylvanoides sylvanoides|24127A11|Canada:British Columbia|2003
Ochlodes sylvanoides sylvanoides|24011G10|Canada:British Columbia|1933
Ochlodes sylvanoides sylvanoides|24127A09|Canada:British Columbia|1985
Ochlodes sylvanoides sylvanoides|24127A12|Canada:British Columbia|1985
Ochlodes sylvanoides sylvanoides|24127B10|USA:ID,Custer Co.|1975
Ochlodes sylvanoides sylvanoides|20063G06|USA:WA,Chelan Co.|2006
Ochlodes sylvanoides sylvanoides|24126B07|USA:MT,Ravalli Co.|1980
Ochlodes sylvanoides sylvanoides|24126B08|USA:MT,Missoula Co.|1988
Ochlodes sylvanoides sylvanoides|24127B03|USA:ID,Canyon Co.|1976
Ochlodes sylvanoides sylvanoides|24014A02|Canada:British Columbia|1998
Ochlodes sylvanoides sylvanoides|24126B05|USA:MT,Flathead Co.|1975
Ochlodes sylvanoides sylvanoides|24014A04|Canada:British Columbia|1953
Ochlodes sylvanoides sylvanoides|24127A10|Canada:British Columbia|1976
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|24014A06|Canada:Alberta|1998
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|24014A07|Canada:Alberta|1996
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|24014A05|Canada:Saskatchewan|1983
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D02|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D03|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D04|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D05|HT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D06|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24126B10|PT|USA:MT,Big Horn Co.|1997
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24126C01|PT|USA:MT,Big Horn Co.|1954
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24126C06|HT|USA:WY,Washakie Co.|1998
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24127A08|PT|USA:SD,Butte Co.|1983
Ochlodes napa kaibab|21113C06|PT|USA:AZ,Coconino Co.|2021
Ochlodes napa kaibab|21113C07|HT|USA:AZ,Coconino Co.|2021
Ochlodes napa kaibab|24126B06|USA:UT,Kane Co.|1987
Ochlodes napa kaibab|24126C07|USA:WY,Teton Co.|1972
Ochlodes napa kaibab|24126D11|USA:UT,Kane Co.|1987
Ochlodes napa kaibab|24126D03|USA:UT,Beaver Co.|1989
Ochlodes napa kaibab|24126C02|USA:WY,Park Co.|2000
Ochlodes napa kaibab|24126D10|USA:UT,Kane Co.|1987
Ochlodes napa kaibab|24126E03|USA:UT,Emery Co.|1980
Ochlodes napa kaibab|24126D07|USA:UT,Emery Co.|1997
Ochlodes napa napa|20045F09|USA:UT,Grand Co.|2016
Ochlodes napa napa|24126G10|USA:CO,Mineral Co.|1934
Ochlodes napa napa|24126G11|USA:CO,Archuleta Co.|1934
Ochlodes napa napa|24126H02|USA:CO,Dolores Co.|1985
Ochlodes napa napa|20045F10|USA:UT,San Juan Co.|2020
Ochlodes napa napa|24126E05|USA:NM,Rio Arriba Co.|1953
Ochlodes napa napa|24126H08|USA:CO,Eagle Co.|1984
Ochlodes napa napa|24126C04|USA:WY,Carbon Co.|1981
Ochlodes napa napa|24126E06|USA:NM,Rio Arriba Co.|1953
Ochlodes napa napa|24126G12|USA:CO,Pitkin Co.|1934
Ochlodes napa napa|24126H11|USA:CO,Grand Co.|1980
Ochlodes napa napa|24126D06|USA:UT,Uintah Co.|1960
Ochlodes napa napa|24126H03|USA:CO,Gunnison Co.|1957
Ochlodes napa napa|24126H04|USA:CO,Routt Co.|1959
Ochlodes napa napa|24126G06|USA:CO,Garfield Co.|1988
Ochlodes napa napa|24126H07|USA:CO,Rio Blanco Co.|1984
Ochlodes napa napa|15098B02|NT|USA:CO,Clear Creek Co.|old
Ochlodes napa napa|24126H12|USA:CO,Boulder Co.|1952
Ochlodes napa napa|24127A01|USA:CO,El Paso Co.|1931
Ochlodes napa napa|24127A05|USA:CO,El Paso Co.|1986
Ochlodes napa napa|24127A03|USA:CO,Gilpin Co.|1996
Ochlodes napa napa|24127A06|USA:CO,Gilpin Co.|1996
Ochlodes napa napa|PAO263|USA:CO,Larimer Co.|2016
Ochlodes napa napa|24126G07|USA:CO,Grand Co.|1980
0.005

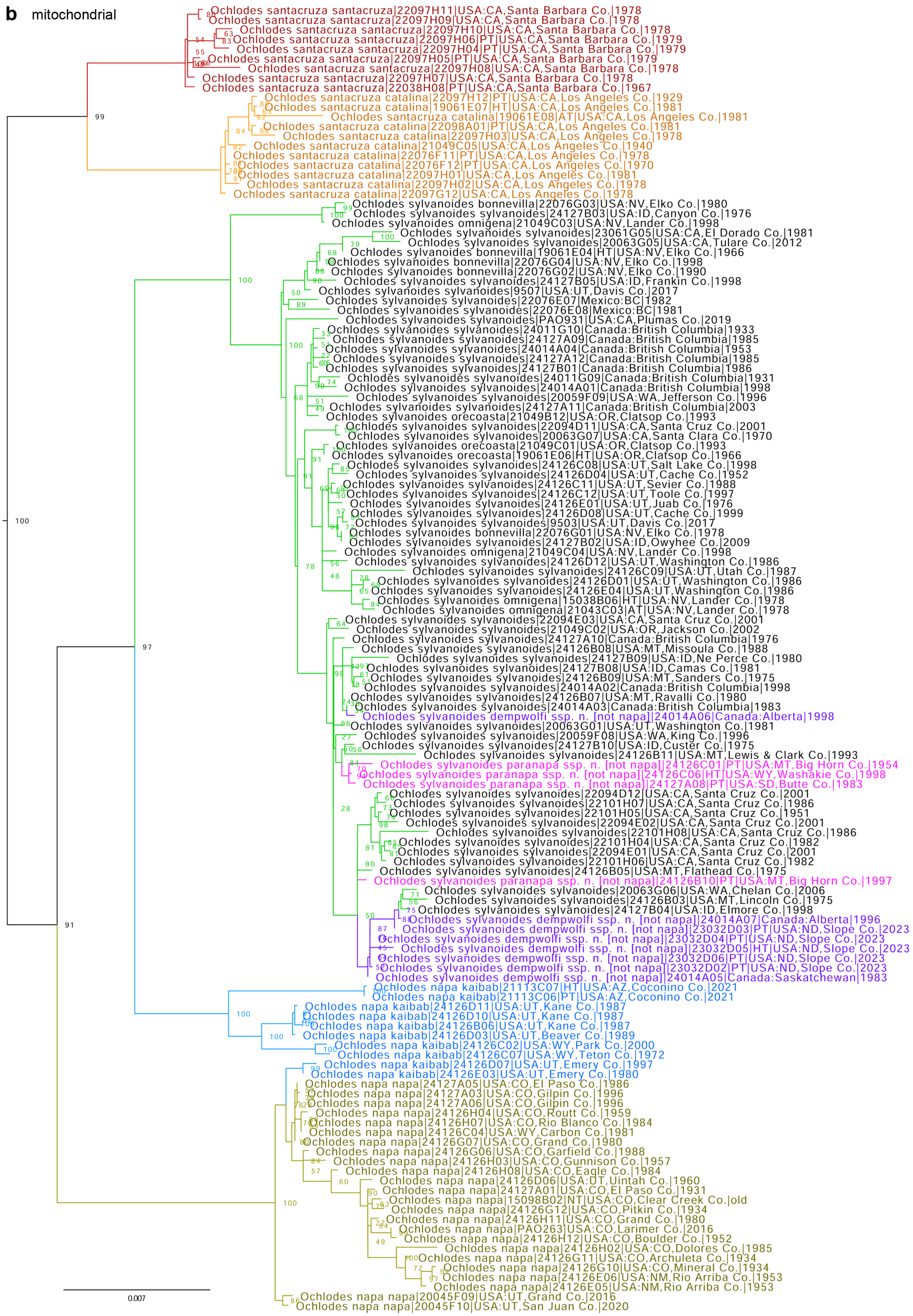

b
mitochondrial
Ochlodes santacruza santacruza|22097H11|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H09|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H10|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H06|PT|USA:CA,Santa Barbara Co.|1979
Ochlodes santacruza santacruza|22097H04|PT|USA:CA,Santa Barbara Co.|1979
Ochlodes santacruza santacruza|22097H05|PT|USA:CA,Santa Barbara Co.|1979
Ochlodes santacruza santacruza|22097H08|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22097H07|USA:CA,Santa Barbara Co.|1978
Ochlodes santacruza santacruza|22038H08|PT|USA:CA,Santa Barbara Co.|1967
Ochlodes santacruza catalina|22097H12|PT|USA:CA,Los Angeles Co.|1929
Ochlodes santacruza catalina|19061E07|HT|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|19061E08|AT|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|22098A01|PT|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|22097H03|USA:CA,Los Angeles Co.|1978
Ochlodes santacruza catalina|21049C05|USA:CA,Los Angeles Co.|1940
Ochlodes santacruza catalina|22076F11|PT|USA:CA,Los Angeles Co.|1978
Ochlodes santacruza catalina|22076F12|PT|USA:CA,Los Angeles Co.|1970
Ochlodes santacruza catalina|22097H01|USA:CA,Los Angeles Co.|1981
Ochlodes santacruza catalina|22097H02|USA:CA,Los Angeles Co.|1978
Ochlodes santacruza catalina|22097G12|USA:CA,Los Angeles Co.|1978
Ochlodes sylvanoides bonnevilla|22076G03|USA:NV,Elko Co.|1980
Ochlodes sylvanoides sylvanoides|24127B03|USA:ID,Canyon Co.|1976
Ochlodes sylvanoides omnigena|21049C03|USA:NV,Lander Co.|1998
Ochlodes sylvanoides sylvanoides|23061G05|USA:CA,El Dorado Co.|1981
Ochlodes sylvanoides sylvanoides|20063G05|USA:CA,Tulare Co.|2012
Ochlodes sylvanoides bonnevilla|19061E04|HT|USA:NV,Elko Co.|1966
Ochlodes sylvanoides bonnevilla|22076G04|USA:NV,Elko Co.|1998
Ochlodes sylvanoides bonnevilla|22076G02|USA:NV,Elko Co.|1990
Ochlodes sylvanoides sylvanoides|24127B05|USA:ID,Frankin Co.|1998
Ochlodes sylvanoides sylvanoides|9507|USA:UT,Davis Co.|2017
Ochlodes sylvanoides sylvanoides|22076E07|Mexico:BC|1982
Ochlodes sylvanoides sylvanoides|22076E08|Mexico:BC|1981
Ochlodes sylvanoides sylvanoides|PAO931|USA:CA,Plumas Co.|2019
Ochlodes sylvanoides sylvanoides|24011G10|Canada:British Columbia|1933
Ochlodes sylvanoides sylvanoides|24127A09|Canada:British Columbia|1985
Ochlodes sylvanoides sylvanoides|24014A04|Canada:British Columbia|1953
Ochlodes sylvanoides sylvanoides|24127A12|Canada:British Columbia|1985
Ochlodes sylvanoides sylvanoides|24127B01|Canada:British Columbia|1986
Ochlodes sylvanoides sylvanoides|24011G09|Canada:British Columbia|1931
Ochlodes sylvanoides sylvanoides|24014A01|Canada:British Columbia|1998
Ochlodes sylvanoides sylvanoides|20059F09|USA:WA,Jefferson Co.|1996
Ochlodes sylvanoides sylvanoides|24127A11|Canada:British Columbia|2003
Ochlodes sylvanoides orecoasta|21049B12|USA:OR,Clatsop Co.|1993
Ochlodes sylvanoides sylvanoides|22094D11|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|20063G07|USA:CA,Santa Clara Co.|1970
Ochlodes sylvanoides orecoasta|21049C01|USA:OR,Clatsop Co.|1993
Ochlodes sylvanoides orecoasta|19061E06|HT|USA:OR,Clatsop Co.|1966
Ochlodes sylvanoides sylvanoides|24126C08|USA:UT,Salt Lake Co.|1998
Ochlodes sylvanoides sylvanoides|24126D04|USA:UT,Cache Co.|1952
Ochlodes sylvanoides sylvanoides|24126C11|USA:UT,Sevier Co.|1988
Ochlodes sylvanoides sylvanoides|24126C12|USA:UT,Toole Co.|1997
Ochlodes sylvanoides sylvanoides|24126E01|USA:UT,Juab Co.|1976
Ochlodes sylvanoides sylvanoides|24126D08|USA:UT,Cache Co.|1999
Ochlodes sylvanoides sylvanoides|9503|USA:UT,Davis Co.|2017
Ochlodes sylvanoides bonnevilla|22076G01|USA:NV,Elko Co.|1978
Ochlodes sylvanoides sylvanoides|24127B02|USA:ID,Owyhee Co.|2009
Ochlodes sylvanoides omnigena|21049C04|USA:NV,Lander Co.|1998
Ochlodes sylvanoides sylvanoides|24126D12|USA:UT,Washington Co.|1986
Ochlodes sylvanoides sylvanoides|24126C09|USA:UT,Utah Co.|1987
Ochlodes sylvanoides sylvanoides|24126D01|USA:UT,Washington Co.|1986
Ochlodes sylvanoides sylvanoides|24126E04|USA:UT,Washington Co.|1986
Ochlodes sylvanoides omnigena|15038B06|HT|USA:NV,Lander Co.|1978
Ochlodes sylvanoides omnigena|21043C03|AT|USA:NV,Lander Co.|1978
Ochlodes sylvanoides sylvanoides|22094E03|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|21049C02|USA:OR,Jackson Co.|2002
Ochlodes sylvanoides sylvanoides|24127A10|Canada:British Columbia|1976
Ochlodes sylvanoides sylvanoides|24126B08|USA:MT,Missoula Co.|1988
Ochlodes sylvanoides sylvanoides|24127B09|USA:ID,Ne Perce Co.|1980
Ochlodes sylvanoides sylvanoides|24127B08|USA:ID,Camas Co.|1981
Ochlodes sylvanoides sylvanoides|24126B09|USA:MT,Sanders Co.|1975
Ochlodes sylvanoides sylvanoides|24014A02|Canada:British Columbia|1998
Ochlodes sylvanoides sylvanoides|24126B07|USA:MT,Ravalli Co.|1980
Ochlodes sylvanoides sylvanoides|24014A03|Canada:British Columbia|1983
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|24014A06|Canada:Alberta|1998
Ochlodes sylvanoides sylvanoides|20063G01|USA:UT,Washington Co.|1981
Ochlodes sylvanoides sylvanoides|20059F08|USA:WA,King Co.|1996
Ochlodes sylvanoides sylvanoides|24127B10|USA:ID,Custer Co.|1975
Ochlodes sylvanoides sylvanoides|24126B11|USA:MT,Lewis & Clark Co.|1993
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24126C01|PT|USA:MT,Big Horn Co.|1954
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24126C06|HT|USA:WY,Washakie Co.|1998
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24127A08|PT|USA:SD,Butte Co.|1983
Ochlodes sylvanoides sylvanoides|22094D12|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|22101H07|USA:CA,Santa Cruz Co.|1986
Ochlodes sylvanoides sylvanoides|22101H05|USA:CA,Santa Cruz Co.|1951
Ochlodes sylvanoides sylvanoides|22094E02|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|22101H08|USA:CA,Santa Cruz Co.|1986
Ochlodes sylvanoides sylvanoides|22101H04|USA:CA,Santa Cruz Co.|1982
Ochlodes sylvanoides sylvanoides|22094E01|USA:CA,Santa Cruz Co.|2001
Ochlodes sylvanoides sylvanoides|22101H06|USA:CA,Santa Cruz Co.|1982
Ochlodes sylvanoides sylvanoides|24126B05|USA:MT,Flathead Co.|1975
Ochlodes sylvanoides paranapa ssp. n. [not napa]|24126B10|PT|USA:MT,Big Horn Co.|1997
Ochlodes sylvanoides sylvanoides|20063G06|USA:WA,Chelan Co.|2006
Ochlodes sylvanoides sylvanoides|24126B03|USA:MT,Lincoln Co.|1975
Ochlodes sylvanoides sylvanoides|24127B04|USA:ID,Elmore Co.|1998
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|24014A07|Canada:Alberta|1996
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D03|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D04|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D05|HT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D06|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|23032D02|PT|USA:ND,Slope Co.|2023
Ochlodes sylvanoides dempwolfi ssp. n. [not napa]|24014A05|Canada:Saskatchewan|1983
Ochlodes napa kaibab|21113C07|HT|USA:AZ,Coconino Co.|2021
Ochlodes napa kaibab|21113C06|PT|USA:AZ,Coconino Co.|2021
Ochlodes napa kaibab|24126D11|USA:UT,Kane Co.|1987
Ochlodes napa kaibab|24126D10|USA:UT,Kane Co.|1987
Ochlodes napa kaibab|24126B06|USA:UT,Kane Co.|1987
Ochlodes napa kaibab|24126D03|USA:UT,Beaver Co.|1989
Ochlodes napa kaibab|24126C02|USA:WY,Park Co.|2000
Ochlodes napa kaibab|24126C07|USA:WY,Teton Co.|1972
Ochlodes napa kaibab|24126D07|USA:UT,Emery Co.|1997
Ochlodes napa kaibab|24126E03|USA:UT,Emery Co.|1980
Ochlodes napa napa|24127A05|USA:CO,El Paso Co.|1986
Ochlodes napa napa|24127A03|USA:CO,Gilpin Co.|1996
Ochlodes napa napa|24127A06|USA:CO,Gilpin Co.|1996
Ochlodes napa napa|24126H04|USA:CO,Routt Co.|1959
Ochlodes napa napa|24126H07|USA:CO,Rio Blanco Co.|1984
Ochlodes napa napa|24126C04|USA:WY,Carbon Co.|1981
Ochlodes napa napa|24126G07|USA:CO,Grand Co.|1980
Ochlodes napa napa|24126G06|USA:CO,Garfield Co.|1988
Ochlodes napa napa|24126H03|USA:CO,Gunnison Co.|1957
Ochlodes napa napa|24126H08|USA:CO,Eagle Co.|1984
Ochlodes napa napa|24126D06|USA:UT,Uintah Co.|1960
Ochlodes napa napa|24127A01|USA:CO,El Paso Co.|1931
Ochlodes napa napa|15098B02|NT|USA:CO,Clear Creek Co.|old
Ochlodes napa napa|24126G12|USA:CO,Pitkin Co.|1934
Ochlodes napa napa|24126H11|USA:CO,Grand Co.|1980
Ochlodes napa napa|PAO263|USA:CO,Larimer Co.|2016
Ochlodes napa napa|24126H12|USA:CO,Boulder Co.|1952
Ochlodes napa napa|24126H02|USA:CO,Dolores Co.|1985
Ochlodes napa napa|24126G11|USA:CO,Archuleta Co.|1934
Ochlodes napa napa|24126G10|USA:CO,Mineral Co.|1934
Ochlodes napa napa|24126E06|USA:NM,Rio Arriba Co.|1953
Ochlodes napa napa|24126E05|USA:NM,Rio Arriba Co.|1953
Ochlodes napa napa|20045F09|USA:UT,Grand Co.|2016
Ochlodes napa napa|20045F10|USA:UT,San Juan Co.|2020
100
99
97
91
0.007

Fig. 20 (see the previous two pages). Phylogenetic trees of the *Ochlodes sylvanoides* group species constructed from protein-coding regions in: **a)** the nuclear genome (autosomes), based on 312,678 positions, and **b)** the mitochondrial genome. Different subspecies are colored differently: *O. santacruza santacruza* (red), *O. santacruza catalina* (orange), *O. sylvanoides sylvanoides* including *orecoasta*, *bonnevilla*, and *omnigena* (green, labeled in black), *O. sylvanoides dempwolfi* **ssp. n.** (violet), *O. sylvanoides paranapa* **ssp. n.** (magenta), *O. napa kaibab* (blue), and *O. napa napa* (olive). Bootstrap values strongly supporting (>90%) the two new subspecies clades are highlighted in yellow in (a).

---

and *Ochlodes sylvanoides omnigena* Austin, 1998 (type locality in USA: Nevada, Lander Co.). While we do not propose synonymizing these subspecies, studies of their distinction are beyond the scope of this work and will be addressed in future research. Here, all these populations combined are shown as green squares in Fig. 19.

Ochlodes (*Ochluma*) *sylvanoides paranapa* Grishin, new subspecies

http://zoobank.org/ADC1FE05-7B71-4C9D-B788-64E7A2F82FE2

(Figs. 19 part, 20 part, 21b–d)

Definition and diagnosis. Genomic analysis reveals that populations from the northeastern part of the range, historically identified as *Ochlodes napa* (W. H. Edwards, 1865) (type locality in USA: Colorado, Clear Creek Co.), are not in the same clade with this species but instead belong to *Ochlodes* (*Ochluma*) *sylvanoides* (Boisduval, 1852) (type locality in USA: California, Plumas Co.), being genetically differentiated from others at the subspecies level (Fig. 20a magenta and violet). While their COI barcodes (or mitogenomes, Fig. 20b) do not consistently differ, these populations together form a distinct moderately supported (88% bootstrap value) clade in the nuclear genome tree (Fig. 20a) that partitions into two more strongly supported subclades: northeastern (Fig. 20a violet, 93% bootstrap) and southwestern (Fig. 20a magenta, 100% bootstrap). The southwestern clade is most strongly differentiated genetically (longer branch) and supported statistically (100%) encompassing specimens from a wide geographical area spanning three states (Montana, Wyoming, and South Dakota) (Fig. 19 magenta). In addition to differences in DNA, these specimens are characterized by phenotypic differences from the nominate subspecies (due to which they were misidentified as *O. napa*), and, therefore, represent a new subspecies of *O. sylvanoides*. This new subspecies keys to "*Ochlodes sylvanoides napa*" (M.19.2(c)) in Evans (1955), but differs from it and other relatives by the following combination of characters: paler below, with a weaker pattern (Fig. 21b–d) than typical for the nominate subspecies (Fig. 21a), i.e., brown borders above are narrower, paler, and more diffuse at the edges; the ventral side is yellower (instead of redder) and usually with a more weakly defined brownish or reddish pattern, but typically more prominent than in *O. napa* (Fig. 21g–j) postdiscal pale bands on both wings; dorsally slightly darker than *O. napa*, with a stronger contrast between brown and orange areas and broader dorsal hindwing brown margins with more strongly developed brown overscaling over the orange areas between inverted brown triangles. Due to extensive individual variation in wing patterns, this subspecies is best identified by DNA, with diagnostic base pairs in the nuclear genome: aly275184.2.3:C501T, aly275184.2.3:C885T, aly2085.1.10:A675G, aly2085.1.10:C678T, aly1846.1.1:C185A; and the COI barcode does not distinguish this subspecies from others.

Barcode sequence of the holotype. Sample NVG-24126C06, GenBank PV892290, 658 base pairs:

```
AACTTTATACTTTATTTTTGGTATTTGAGCAGGAATATTAGGAACTTCTTTAAGTTTATTAATTCGTACAGAATTAGGTAATCCAGGATCTTTAATTGGCGATGACCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGGAGCTCCTGATATAGCATTTCCTCGAA
TAAATAATATAAGATTTTGAATATTACCTCCTTCATTAACATTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGTTGAACAGTATATCCTCCTTTATCTTCTAATATTGC
TCACCAAGGATCTTCTGTTGATTTAGCAATTTTTTCTCTTCATTTAGCTGGTATTTCATCTATTCTAGGAGCTATTAATTTTATTACAACAATTATCAATATACGAATTAAAAACTTATCA
TTTGATCAAATACCCTTATTTGTATGATCAGTAGGTATTACAGCATTATTATTATTATTATCTTTACCTGTATTAGCAGGTGCTATTACAATATTACTTACTGATCGAAATTTAAATACTT
CTTTTTTTGATCCAGCAGGAGGAGGAGATCCAATTTTATATCAACATTTATTT
```

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 21b, bears the following five printed (handwritten text in italics) rectangular labels, four white: [WY WASHAKIE Co. | T47N R86W S5 | Tensleep Reserve | 6400' 11-12.viii.98], [Allyn Museum | Acc. 1998-15], [Ochlodes sylvanoides | (Boisduval, 1852) ♂ | Det. S.R. Steinhauser], [DNA sample ID: | NVG-24126C06 | c/o Nick V.

Grishin], and one red [HOLOTYPE ♂ | Ochlodes sylvanoides | paranapa Grishin]. **Paratypes:** 4♂♂ and 1♀ from USA in MGCL: <u>Montana</u>, Big Horn Co.: 1♂ NVG-24126B10 foothills of Pryor Mts., along Sage Creek, 5550’, 45.2272, −108.5856, 13-Aug-1997, Chuck & Chris Harp leg. (Fig. 21c) and 1♀ NVG-24126B12 25 mi SW of Lodge Grass, 6-Sep-1971, Jack Harry leg.; 1♂ NVG-24126C01 <u>Wyoming</u>, Big Horn Co., Red Grade Spring, 5000’, 13-Aug-1954; and <u>South Dakota</u>: 1♂ NVG-24127A08 Butte Co., USH212 at mi 17, ca. 2 mi E of Belle Fourche, 3-Aug-1983, D. L. Eiler leg. (Fig. 21d) and 1♂ NVG-24127A07 Lawrence Co., no locality details, 27-Aug-1969, M. L. May.

Type locality. USA: Wyoming, Washakie Co., Bighorn Mountains, Tensleep Preserve, elevation 6400 ft.

Etymology. The name reflects a superficial resemblance between *O. napa* and this new subspecies of *O. sylvanoides*, as it parallels *O. napa* but occurs at more northern latitudes. The name is treated as a noun in apposition.

Distribution. Currently known from east of the main Rocky Mountain chain in Montana and Wyoming, and from South Dakota.

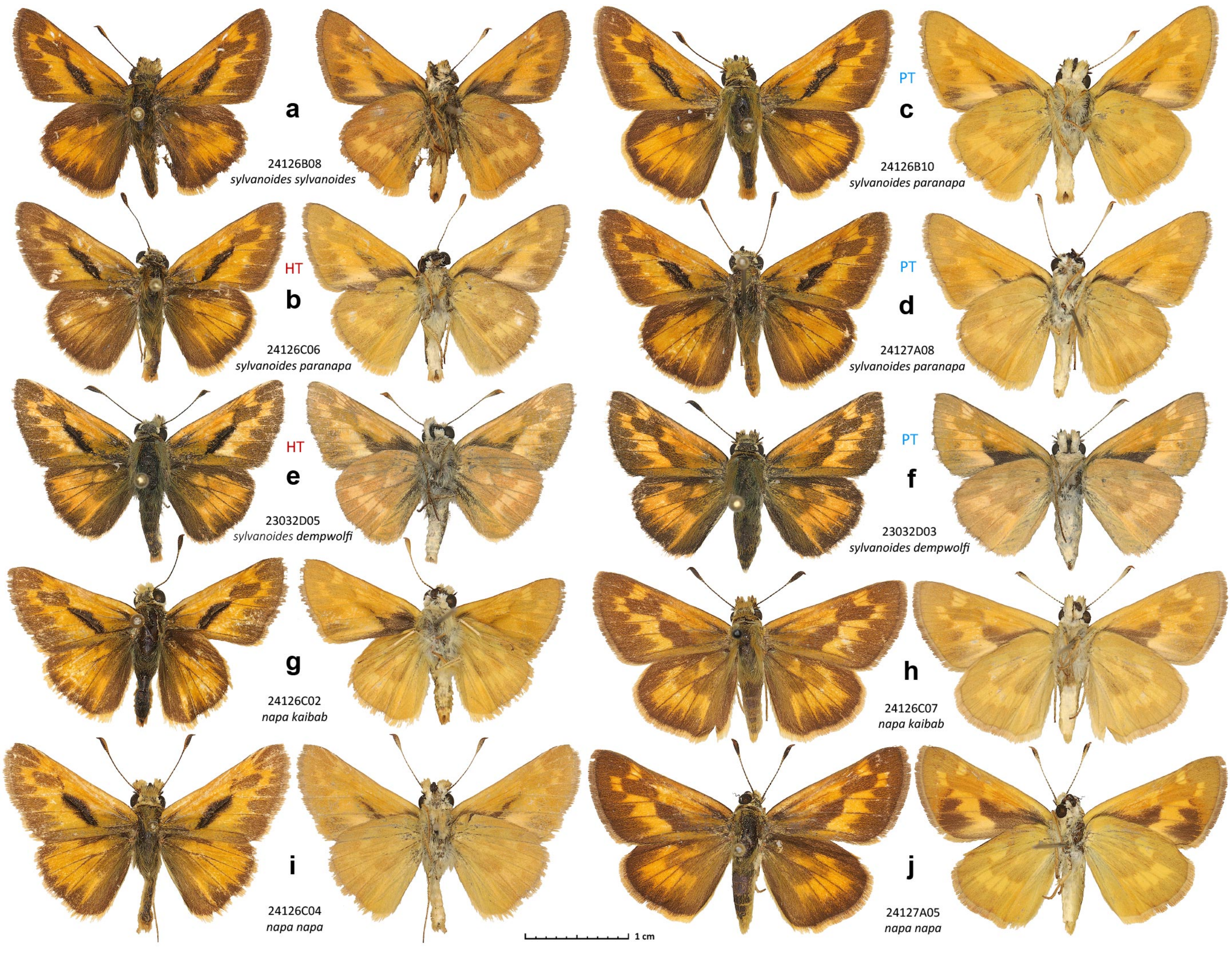


Fig. 21. Selected specimens of *Ochlodes* (*Ochluma*) *sylvanoides* group from USA in dorsal (left) and ventral (right) views, data in text or below [MGCL unless indicated]: **a)** *O. sylvanoides sylvanoides* ♂ NVG-24126B08 MT, Missoula Co., Nine Mile Creek, 8-Aug-1988, S. Kohler leg.; **b–d)** *O. sylvanoides paranapa* **ssp. n.**: **b)** holotype ♂ NVG-24126C06 WY, Washakie Co. and paratypes: **c)** ♂ NVG-24126B10 MT, Big Horn Co. and **d)** ♂ NVG-24127A08 SD, Butte Co.; **e–f)** *O. sylvanoides dempwolfi* **ssp. n.** from ND, Slope Co.: **e)** holotype ♂ NVG-23032D05 and **f)** paratype ♀ NVG-23032D03 [WRD]; **g–h)** *O. napa kaibab* from WY: **g)** ♂ NVG-24126C02 Park Co., Front, Dead Indian Hill, 6400’, 3-Aug-2000, E. H. Williams leg. and **h)** ♀ NVG-24126C07 Teton Co., Yellowstone National Park, Mammoth Hot Springs, 16-Aug-1972, D. L. Lindsley leg.; **i–j)** *O. napa napa*: **i)** ♂ NVG-24126C04 WY, Carbon Co., Saratoga, 3-Aug-1981, D. L. Eiler leg. and **j)** ♀ NVG-24127A05 CO, El Paso Co., Garden of Gods, 12-18-Jul-1986, J. Hyatt leg.

Ochlodes (*Ochluma*) *sylvanoides dempwolfi* Grishin, new subspecies

http://zoobank.org/F27AE8D7-F5DA-4B7F-B444-A3343470021A

(Figs. 19 part, 20 part, 21e–f)

Definition and diagnosis. Genomic analysis reveals that populations from the northeastern part of the range, historically identified as *Ochlodes napa* (W. H. Edwards, 1865) (type locality in USA: Colorado, Clear Creek Co.), are not in the same clade with this species but instead belong to *Ochlodes* (*Ochluma*) *sylvanoides* (Boisduval, 1852) (type locality in USA: California, Plumas Co.), being genetically differentiated from others at the subspecies level (Fig. 20a violet and magenta). While their COI barcodes (or mitogenomes, Fig. 20b) do not consistently differ, these populations together form a distinct moderately supported (88% bootstrap value) clade in the nuclear genome tree (Fig. 20a) that partitions into two more strongly supported subclades: northeastern (Fig. 20a violet, 93% bootstrap) and southwestern (Fig. 20a magenta, 100% bootstrap). The southwestern clade represents a new subspecies described above. Its sister, the northeastern clade, includes specimens from a wider geographical area from southern Alberta and Saskatchewan in Canada, to North Dakota in the U.S. (Fig. 19 violet). These specimens are characterized by phenotypic and genetic differences from other subspecies of *O. sylvanoides* and, therefore, represent a new subspecies. This new subspecies keys to "*Ochlodes sylvanoides napa*" (M.19.2(c)) in Evans (1955), but differs from it and other relatives by being intermediate in appearance between the nominate and the new subspecies described above or *O. napa*: both sides of wings are paler than a typical nominate specimen (Fig. 21a), but the marginal brown areas above are also rather wide and more prominently scalloped, with sharply defined edges on the forewing and more diffuse on the hindwing; the ventral hindwing with muted reddish-brown ground color, which is less bright than in the nominate subspecies, but darker than in *O. sylvanoides paranapa* **ssp. n.** (Fig. 21b–d) and both darker and redder than in *O. napa* (Fig. 21g–j); and the dorsal pattern of orange bands of spots is unique in terms of each spot being slightly yellower in the middle and more orange around, instead of uniformly toned, as in other subspecies. Due to extensive individual variation in wing patterns, this subspecies is best identified by DNA, with diagnostic base pairs in the nuclear genome: aly848.2.60: G48A, aly25.8.2:C84T, aly25.8.2:C87T, aly935.4.26:G54A, aly935.4.26:C67T; and the COI barcode does not distinguish this subspecies from others.

Barcode sequence of the holotype. Sample NVG-23032D05, GenBank PV892291, 658 base pairs:

```
AACTTTATACTTTATTTTTGGTATTTGAGCAGGAATATTAGGAACTTCTTTAAGTTTATTAATTCGTACAGAATTAGGTAATCCAGGATCTTTAATTGGCGATGACCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGGAGCTCCTGATATAGCATTTCCTCGAA
TAAATAATATAAGATTTTGAATATTACCTCCTTCATTAACATTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGTTGAACAGTATATCCTCCTTTATCTTCTAATATTGC
TCACCAAGGATCTTCTGTTGATTTAGCAATTTTTTCTCTTCATTTAGCTGGTATTTCATCTATTCTAGGAGCTATTAATTTTATTACAACAATTATCAATATACGAATTAAAAACTTATCA
TTTGATCAAATACCCTTATTTGTATGATCAGTAGGTATTACAGCATTATTATTATTATTATCTTTACCTGTATTAGCAGGTGCTATTACAATATTACTTACTGATCGAAATTTAAATACTT
CTTTTTTTGATCCAGCAGGAGGAGGAGATCCAATTTTATATCAACATTTATTT
```

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity Collection, Gainesville, FL, USA (MGCL), illustrated in Fig. 21e, bears the following five printed rectangular labels, four white: [ND: Slope Co. elev 2560' | East River Road, approximately | 1.75 miles from Burning Coal | Vein Campground 46° 35' 51.1" | N, 103° 27' 24.5" W August 23, | 2023 Leg: W. R. Dempwolf], [Ochlodes sylvanoides | napa | ♂ | Coll of: W R Dempwolf], [DNA sample ID: | NVG-23032D05 | c/o Nick V. Grishin], [WRD 23,554], and one red [HOLOTYPE ♂ | Ochlodes sylvanoides | dempwolfi Grishin]. **Paratypes:** 1♂ and 3♀♀: from USA, North Dakota, Slope Co. data as the holotype except as indicated, [WRD]: 1♂ NVG-23032D06, WRD 23555 22-Aug-2023, 1♀ NVG-23032D02, WRD 23551; 1♀ NVG-23032D03; WRD 23552 (Fig. 21f); and 1♀ NVG-23032D04, WRD 23553 East River Road, ca. 18 mi south of Medora, 2647', 46.698861, −103.487417, 22-Aug-2023.

Other specimens: Due to genetic similarity (Fig. 20), we currently attribute the following three sequenced specimens from Canada in the CNC to this subspecies but exclude them from the type series because they exhibit some differences compared to the population at the type locality: Alberta: 1♂ NVG-24014A07, CNCLEP 00169968 Taber, 49.7873, −112.1490, 16-Aug-1996, T. Pike leg. and 1♀ NVG-24014A06, CNCLEP 00169953 Hwy 880 & US border, 49.0000, −111.2667, 16-Aug-1998, R. A. Layberry leg. and 1♂ NVG-24014A05, CNCLEP 00169950 Saskatchewan, Val Marie, 49.2464, −107.7283, 10-Aug-1983, R. Hooper leg.

Type locality. USA: North Dakota, Slope Co., East River Road, ca. 1.75 mi from Burning Coal Vein Campground, elevation 2560’, GPS 46.5975, −103.4568.

Etymology. The name, a noun in the genitive case, honors Bill Dempwolf, the collector of the type series and a friend of the author. An exceptional lepidopterist, Bill is recognized for meticulously curating and assembling one of the finest collections. His generosity has been instrumental in our genomic studies, manifested through both dedicated specimen collection for our lab research and open access to his entire collection for leg sampling and sequencing. His profound contributions to our projects are highly significant and greatly appreciated.

Distribution. Northeastern parts of the range, east of the Rockies from Alberta and Saskatchewan in Canada to North Dakota in the U.S.

Thespieus grandosul Grishin, new species

http://zoobank.org/8E47C852-82A8-4841-B2E0-85DE20E52512

(Figs. 22 part, 23)

Definition and diagnosis. Genomic analysis reveals that a specimen from Rio Grande do Sul, Brazil, is sister in the nuclear genome (autosomes) tree to the sympatric *Thespieus ethemides* (Burmeister, 1878) (type locality in Argentina: Corrientes), but is genetically differentiated from it at the species level (Fig. 22a); e.g., their COI barcodes differ by 3.8% (25 bp), and, therefore, represents a new species. In the Z chromosome and the mitochondrial genome trees (Fig. 22b, c), this new species is sister to *Thespieus dalman* (Latreille, [1824]) (type locality in Brazil) and is phenotypically more similar to it, thus keying to *T. dalman* (O.7.4) in Evans (1955), but differs from both *T. dalman* and *T. ethemides* by larger hyaline spots, e.g., on the hindwing, the spot in the cell CuA_1-CuA_2 is longer than wide and starts from the base of the vein CuA_1, and the base of the cell CuA_1-CuA_2 (basad of the spot) is paler brown, not nearly black as the rectangle distad of the spot. Due to its cryptic nature and unexplored individual variation, this species is best identified by DNA, with diagnostic base pairs in the nuclear genome: aly159.13.2:G66A, aly1019.14.13:G165A, aly383.29.15:T210C, aly536.75.1:C2445T, aly3507.14.4:C1098T, aly3446.4.1:A21A (not T), aly638.29.3:G120G (not T), aly994.6.3:C132C (not T), aly2275.10.7:T120T (not C), aly525.73.10:G153G (not A); and the COI barcode: T46T, C59C, T460T, T514C, A628G.

Barcode sequence of the holotype. Sample NVG-23109G03, GenBank PV892292, 658 base pairs:

```
AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATATTAGGAACTTCATTAAGATTACTAATTCGTACAGAATTAGGTAATCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTCGGAAATTGATTAATCCCATTAATATTAGGAGCCCCTGATATAGCTTTTCCTCGAA
TAAATAATATAAGATTTTGAATATTACCCCCCTCTTTAACATTATTAATTTCAAGAAGAATTGTAGAAAATGGTGCAGGAACTGGATGAACAGTTTATCCACCTTTATCTTCTAATATTGC
TCATCAAGGATCTTCAGTAGATTTAGCAATCTTTTCTCTTCATTTAGCTGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTACAACAATTATTAACATACGAATTAAAAATTTATCA
TTTGATCAAATACCTTTATTTGTATGATCCGTAGGTATTACAGCATTATTATTACTTTTATCTTTACCTGTATTAGCAGGAGCTATTACTATATTATTAACTGATCGAAATTTAAATACTT
CTTTTTTTGATCCTGCAGGAGGGGGAGATCCAATTTTATATCAACATTTATTT
```

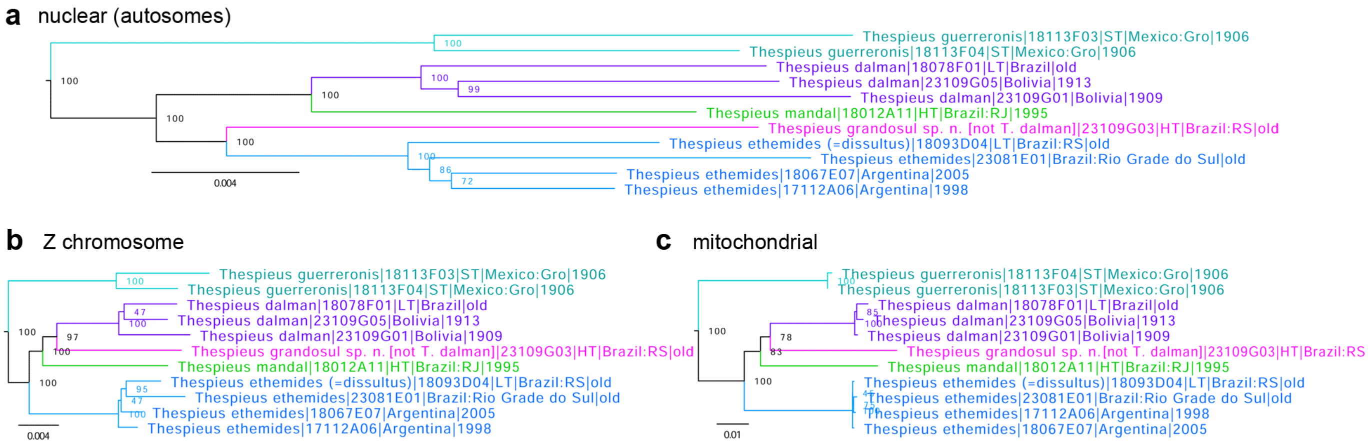


Fig. 22. Phylogenetic trees of *Thespieus dalman* relatives constructed from protein-coding regions in: **a)** the nuclear genome (autosomes), based on 9,744,243 positions, **b)** the Z chromosome, based on 373,431 positions, and **c)** the mitochondrial genome. Different species are colored differently: *T. guerreronis* Dyar, 1913 (cyan), *T. dalman* (purple), *T. mandal* (green), *T. grandosul* **sp. n.** (magenta), and *T. ethemides* (blue).

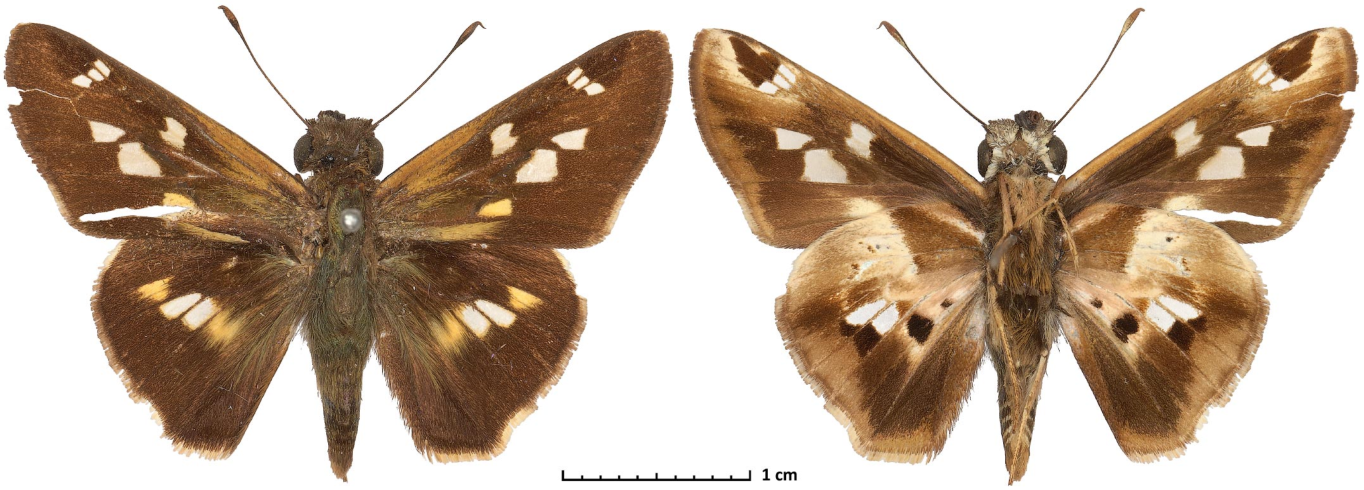


Fig. 23. *Thespieus grandosul* **sp. n.** holotype ♂ NVG-23109G03 in dorsal (left) and ventral (right) views.

Type material. Holotype: ♂ deposited in the Carnegie Museum of Natural History, Pittsburgh, PA, USA (CMNH), illustrated in Fig. 23, bears the following five printed rectangular labels, four white: [Rio Grande | do Sul], [692.], [Lindsay Collection | C. M. Acc. No. 8584], [DNA sample ID: | NVG-23109G03 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Thespieus | grandosul Grishin].

Type locality. Brazil: Rio Grande do Sul.

Etymology. The name is given for the type locality, [Rio] *Gran*[de] + *do* + *Sul*, and is treated as a noun in apposition.

Distribution. Currently known only from the holotype collected in Rio Grande do Sul, Brazil.

ACKNOWLEDGMENTS

We acknowledge Ping Chen and Ming Tang for their excellent technical assistance. We are grateful to David Grimaldi and Courtney Richenbacher (AMNH: American Museum of Natural History, New York, NY, USA), Chris Grinter, Denise Montelongo, David Bettman, Vince Lee, and the late Norm Penny (CAS: California Academy of Sciences, San Francisco, CA, USA), Thomas Pyrcz and Rafał Garlacz (CEPUJ: The Nature Education Center of the Jagiellonian University, Kraków, Poland), Jim Fetzner, Bob Androw, Vanessa Verdecia, Cat Giles, and the late John Rawlins (CMNH: Carnegie Museum of Natural History, Pittsburgh, PA, USA), Chris Schmidt and Christi Jaeger (CNC: Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada), the late Paul Opler, Chuck Harp, and the late Boris Kondratieff (CSUC: Colorado State University Collection, Fort Collins, CO, USA), Crystal Maier and Rebekah Baquiran (FMNH: Field Museum of Natural History, Chicago, IL, USA), Weiping Xie and Giar-Ann Kung (LACM: Los Angeles County Museum of Natural History, Los Angeles, CA, USA), Théo Léger, Christoph L. Häuser, Viola Richter, and Wolfram Mey (MFNB: Museum für Naturkunde, Berlin, Germany), Andrei Sourakov, Andrew D. Warren, Debbie Matthews-Lott, Riley J. Gott, and Keith R. Willmott (MGCL: McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA), Rodolphe Rougerie (MNHP: Muséum National d'Histoire Naturelle, Paris, France), Massimo Terragni, Steffen Pauls, and Wolfgang A. Nässig (SMF: Senckenberg Naturmuseum, Frankfurt, Germany), Hossein Rajaei (SMNS: Staatliches Museum für Naturkunde, Stuttgart, Germany), Mario Cupello, Edward G. Riley, Karen Wright, and John Oswald (TAMU: Texas A&M University Insect Collection, College Station, TX, USA), Alex Wild (TMMC: University of Texas Biodiversity Center, Austin, TX, USA), Jeff Smith and Lynn Kimsey (UCDC: Bohart Museum of Entomology, University of California, Davis, CA, USA), Robert K. Robbins, John M. Burns, and Brian Harris (USNM: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA), and Axel Hausmann, Andreas Segerer, and Ulf Buchsbaum (ZSMC: Zoologische Staatssammlung

München, Germany), for granting access to the collections under their care, sampling specimens, and stimulating discussions; to Ernst Brockmann (Lich, Germany), William R. Dempwolf (WRD: private research collection, Austin, TX, USA), C. Howard Grisham (Huntsville, AL, USA), Robb Hannawacker (UT, USA), the late Paul A. Opler (CO, USA), and Jiro Uehara (Japan) for specimens and leg samples. We are particularly indebted to Bill Dempwolf for expressing his interest in the "high-elevation" populations of *Burnsius communis* in California and for collecting specimens that led to the discovery of a possible overlap zone between the ranges of its two subspecies near Mill Creek Campground in Tuolumne County. This discovery opens opportunities for further research. Bill also collected the type series of *Ochlodes sylvanoides dempwolfi* **ssp. n.** in North Dakota and generously shared these and numerous other specimens for genomic analysis. We are grateful to Keith Edkins for kindly bringing homonymy to our attention, to Norbert Kondla and Cris Guppy for discussions, and to Cris Guppy and Bernard Hermier for critical reviews of the manuscript and many helpful comments and corrections. We are indebted to the California Department of Fish and Game for collecting permit SC13645 and to the Texas Parks and Wildlife Department (Natural Resources Program Director David H. Riskind) for research permit 08-02Rev. We acknowledge the Texas Advanced Computing Center (TACC) at The University of Texas at Austin for providing HPC resources. This study was supported in part by the HHMI Investigator funds and by grants from the National Institutes of Health GM127390 and the Welch Foundation I-1505 to N.V.G.

LITERATURE CITED

Burns, J. M. 2000. *Pyrgus communis* and *Pyrgus albescens* (Hesperiidae: Pyrginae) are separate transcontinental species with variable but diagnostic valves. Journal of the Lepidopterists' Society 54(2): 52–71.

Cong, Q., J. Shen, W. Li, D. Borek, Z. Otwinowski, and N. V. Grishin. **2017**. The first complete genomes of metalmarks and the classification of butterfly families. Genomics 109(5/6): 485–493.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J. F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J. M. Claverie, and O. Gascuel. **2008**. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research 36 (Web Server issue): W465–W469.

Evans, W. H. 1952. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part II. (Groups B, C, D) Pyrginae. Section I. The Trustees of the British Museum (Natural History); London. v + 178 pp., pls. 10–25.

Evans, W. H. 1953. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part III. (Groups E, F, G) Pyrginae. Section 2. The Trustees of the British Museum (Natural History); London. v + 246 pp., pls. 26–53.

Evans, W. H. 1955. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part IV. (Groups H to P) Hesperiinae and Megathyminae. The Trustees of the British Museum (Natural History); London. v + 499 pp., pls. 54–88.

Gascuel, O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Molecular Biology and Evolution 14(7): 685–695.

ICZN [International Commission on Zoological Nomenclature]. **1999**. International code of zoological nomenclature. Fourth edition. International Trust for Zoological Nomenclature; London. xxx + 306.

Minh, B. Q., M. A. Nguyen, and A. von Haeseler. **2013**. Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195.

Pelham, J. P. 2023. Catalogue of the Butterflies of the United States and Canada. Revised 23 February 2023. (http://www.butterfliesofamerica.com/US-Can-Cat.htm). Last accessed 5 July 2025.

Shen, J., Q. Cong, D. Borek, Z. Otwinowski, and N. V. Grishin. **2017**. Complete genome of *Achalarus lyciades*, the first representative of the Eudaminae subfamily of skippers. Current Genomics 18(4): 366–374.

Stål, C. 1866. Hemiptera Africana. Vol. 4. Norstedts; Stockholm. 276 pp.

Steinhauser, S. R. 1981. A revision of the *proteus* group of the genus *Urbanus* Hubner (Lepidoptera: Hesperiidae). Bulletin of the Allyn Museum 62: 1–41.

Steinhauser, S. R. 1987. Notes on the identity of the species-group names in the genera *Urbanus* and *Astraptes* (sensu Evans). Bulletin of the Allyn Museum 111: 1–16.

Warren, A. D. 2005. Butterflies of Oregon: Their Taxonomy, Distribution, and Biology. Lepidoptera of North America 6: 1–408.

Zhang, J., Q. Cong, and N. V. Grishin. **2023a**. Descriptions of one hundred new species of Hesperiidae. Insecta Mundi 1026: 1–115.

Zhang, J., Q. Cong, J. Shen, L. Song, and N. V. Grishin. **2023b**. Genomic analysis reveals new species and subspecies of butterflies. The Taxonomic Report of the International Lepidoptera Survey 11(6): 1–62.

Zhang, J., Q. Cong, J. Shen, L. Song, and N. V. Grishin. **2024**. Genomic analysis reveals hidden species diversity in *Emesis* Fabricius (Lepidoptera: Riodinidae). Insecta Mundi 1082: 1–48.

Zhang, J., Q. Cong, J. Shen, L. Song, and N. V. Grishin. **2025a**. Advancing butterfly systematics through genomic analysis. The Taxonomic Report of the International Lepidoptera Survey 12(5): 1–200.

Zhang, J., Q. Cong, J. Shen, L. Song, and N. V. Grishin. **2025b**. Genomics-based taxonomic refinement of *Emesis* Fabricius (Lepidoptera: Riodinidae). Insecta Mundi 1131: 1–33.

Zhang, J., Q. Cong, J. Shen, L. Song, P. A. Opler, and N. V. Grishin. **2023c**. Additional taxonomic refinements suggested by genomic analysis of butterflies. The Taxonomic Report of the International Lepidoptera Survey 11(1): 1–25.

The Taxonomic Report
is a platinum open access peer-reviewed publication of
The International Lepidoptera Survey (TILS)

The International Lepidoptera Survey is registered as a non-profit Limited Liability Company (LLC) in the state of Virginia, U.S.A. The Taxonomic Report (TTR), ISSN 2643-4776 (print) / ISSN 2643-4806 (online) is published for the purpose of providing a public and permanent scientific record. Articles are peer-reviewed but not necessarily through the anonymous editor-mediated review process. Typically, the authors are encouraged to solicit reviews of their manuscripts from knowledgeable lepidopterists before submission. TTR appears in digital, open-access form, is disseminated as a hardcopy to select institutional repositories, and is available as printed copy upon request at the discretion of authors and/or the editor. Printing and postage charges may apply. An initial run of 25 copies is printed on paper to meet ICZN Code recommendation 8B. All published TTR articles are freely available at the archival TTR website (http://lepsurvey.carolinanature.com/report.html) and via the following digital repositories:

Internet Archive (https://archive.org/)
Biodiversity Heritage Library (https://www.biodiversitylibrary.org)
Zobodat (https://www.zobodat.at/)
Zenodo (https://zenodo.org)
Digital Commons (https://digitalcommons.unl.edu)

TILS Purpose

TILS is devoted to the worldwide collection of Lepidoptera for the purpose of scientific discovery, determination, and documentation, without which there can be no preservation.

TILS Motto

"As a world community, we cannot protect that which we do not know"

Articles for publication are sought

Manuscripts may deal with any area of research on Lepidoptera, including faunal surveys, conservation topics, life histories and foodplant records, matters of nomenclature, descriptions of new taxa, methods, etc. Taxonomic papers are particularly welcome. There are no publication charges for authors. Before submitting a manuscript, email **TTR editor, Harry Pavulaan, 606 Hunton Place NE, Leesburg, VA, 20176, USA** at intlepsurvey@gmail.com (cc: to harrypav@hotmail.com if you do not receive a reply within one week) to initiate discussion on how to best handle your material for publication, and to discuss peer review options.

Visit *The International Lepidoptera Survey* on the World Wide Web at:
http://lepsurvey.carolinanature.com
&
Join the discussion at our list serve on Groups.io at:
https://groups.io/g/TILS
You can subscribe by sending an email to: TILS+subscribe@groups.io
&
Join The International Lepidoptera Survey on Facebook at:
https://www.facebook.com/groups/1072292259768446